FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY
OF HELSINKI

BACTERIAL AGGLUTININS PRESENT IN SEEDS OF SOME REPRESENTATIVES OF THE FAMILY LEGUMINOSEAE

By

SIRKKA ISOTALO

The presence of hemagglutinins in seeds has long been demonstrated. Eisler's and Portheim's study on the subject was published in 1909. They had investigated 99 species of 56 different genera, and found 6 species of the genus *Datura* which agglutinated human blood cells. In the same publication they mention that extracts prepared from seeds of the *Ricinus* and *Datura* species do not agglutinate bacteria, whereas *Phaseolus vulgaris* extract agglutinates *S. typhi* and *V. cholerae* weakly. As the writers gave no detailed description of the technique employed, and as the present writer has not found any other report of bacterial agglutinins present in seeds, a more detailed investigation was considered necessary.

Technique. — The seeds were powdered by hand in a mortar and extracted with saline (1: 9) for two hours at 37° C. After centrifugation the clear extracts were kept for 24 hours in a refrigerator, when a small sediment formed in some of them. For this reason all of them were re-centrifuged, and the clear extracts then obtained were used for the tests. The preparation of bacterial suspensions suitable for the tests was difficult. Broth formed a sediment with most of the seed extracts. Similarly, many extracts precipated even the small amount of agar dissolved in the saline when the agar plate culture was suspended with 2 ml saline and diluted to 1: 15. The bacterial suspensions obtained from agar plate were therefore centrifuged and re-suspended in saline. After centrifugation *Shigel-*

lae Shiga-Kruse, Kruse-Sonne and *Schmitz* autoagglutinated, for
which reason they were cultivated in 200 ml of broth and kept for
48 hours at 37° C. After the purity of the cultures was tested, the
bacterial suspensions were kept for two hours at 56° C. The samples
taken subsequently revealed no growth. The bacterial suspensions
were kept in a refrigerator. After 4 days the broth was poured off
from the broth cultures, and replaced by 100 ml of saline. After
two days the saline was poured off and replaced by addition of new
saline until the turbidity was similar to that of the suspensions
obtained from agar plate. 0.5 ml of extract and 0.5 ml of bacterial
suspension were pipetted into test tubes, kept for an hour in a water
bath at 37° C, and read off by the naked eye.

The bacteria used were:

E. coli str. 1   S. paratyphi A
E. coli str. 2   S. paratyphi B
B. prodigiosus   S. typhi murium
Staphylococcus aureus str. 1   S. marashino
        »   »   str. 2   S. muenchen
Coryneb. dipht.   S. typhi
Vibrio cholerae   Proteus X 19
                                       B. abortus Bangi
Sh. paradys. H 19   Sh.paradys. Kruse-Sonne str. 2
        »   D 118   »   Scmitz VII
        »   Kruse-Sonne str. 1   Sh. dysenteriae Shiga-Kruse

Distinctly negative with all bacterial suspensions were the
following seed extracts (division according to Wettstein):

Mimosacae: Albizzia lophanta
Pàpilionacae:

A. Caesapinioideae: Caesalphina pulcherrima
                    Bauchinia tomentosa
B. Papilionatae:
3. Genisteae: Lupinus angustifolius
                    Laburnum anagyroides
4. Astragaleae: Galega officinalis
                    Astragalus fallatus
                         »   alpinus
8. Trifolieae: Trifolium pratense
                         »   repens
                    Medicago sativa
                    Melilotus albus

9. Vicieae: Vicia tetrasperma
                    »   sativa
                    »   villosa
                    »   sepium
               Pisum sativum
               Ervum hirsutum
               Lathyrus pratensis
                    »   maritimus
                    »   niger
                    »   Clymenum
                    »   tingitanus
                    »   montaneus
               Abrus precatorius

10. Phaseoleae: Phaseolus vulgaris v. communis
                    »   »   v. nanus
               Glycine Soya

The following extracts showed a negative reaction with other bacteria, but behaved differently from other extracts with the bacterial suspensions of *Sh. paradysenteriae Kruse-Sonne* str. 1 *Kruse-Sonne* str. 2 and *Sh. dysenteriae Shiga-Kruse:*

TABLE 1

	Shiga-Kruse	Kruse-Sonne str. 1	Kruse-Sonne str. 2
Genistae:			
Lupinus hybridus	+	−	−
» polyphyllus	−	+	+
Laburnum alpinum	+	+	+
Cytisus sessifolius	−	+	+
Astragaleae:			
Oxytropis campestris	+	+	+
Anthyllis Vulneraria	+	+	+
Coronileae:			
Hedysarum alpinum	−	+	+
Trifolieae:			
Trifolium hybridum	−	+	+
» incarnatum	−	−	+
Medicago scutellata	−	+	+
Vicieae:			
Vicia cracca	−	+	+
Lathyrus silvestris	+	−	−
» vernus	−	−	+

In the test tubes marked + there was a distinct sediment on the bottom and an almost clear layer of liquid. In the control tubes containing bacteria and saline, there was after one hour no sediment, but after two hours it was found on the bottom a sediment, which was similar, also when examined under a magnifying glass, with the sediment obtained in tests in Table 1. Both these sediments differed from the agglutination obtained in the plus control tubes containing *Shigellae* + corresponding immune serum, read after one and two hours. Extracts with saline remained clear at the reading after two hours. As the *Shigellae* strains used have an obvious tendence to auto-agglutination, we do not consider the positive reactions in Table 1 as true agglutination. However, the extracts seemed to accelerate to some extent the sedimentation of the bacteria in question.

SUMMARY

Seed extracts from 40 species of the family *Leguminoseae* were studied in relation to 21 different bacterial suspensions. No definite bacterial agglutinins were found in the seeds.

REFERENCES

1. EISLER, M., and PORTHEIM, L.: Zeitsch. f. Immunitäts f. Orig. 1909:*I*: 157.
2. WETTSTEIN, R.: Handbuch d. Systematischen Botanik, Leipzig, 1935.

AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI
AUS DEM PHARMAZEUTISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ÜBER DEN EINFLUSS DER l-USNINSÄURE AUF DIE EXPERIMENTELLE MEERSCHWEINCHENTUBERKULOSE

Von

RISTO PÄTIÄLÄ, J. PÄTIÄLÄ, SIPI SIINTOLA und P. HEILALA

ÜBERSICHT ÜBER DIE ANTIBIOTISCHEN EIGENSCHAFTEN DER FLECHTEN IM ALLGEMEINEN

Als die seit uralten Zeiten als Volksarzneimittel (Archiv für Volksdichtung in Finnland) gebrauchten Flechten am Anfang des vorigen Jahrhunderts als wichtige Arzneimittel in die Pharmakopöen aufgenommen wurden, wurden sie in erster Linie bei Infektionskrankheiten der Atmungsorgane, wie z.B. Lungentuberkulose, angewandt. Im Jahre 1846 waren in den Pharmakopöen der verschiedenen Länder wenigstens 10 verschiedene Arten von Flechten enthalten und ca. 50 verschiedene Anweisungen zur Arzneibereitung aus denselben wurden darin aufgeführt. Im Jahre 1915 wurde aus der finnischen Pharmakopöe die letzte Flechtendroge *Lichen islandicus*, die Islandflechte, entfernt.

Heutzutage enthalten die Pharmakopöen keine Vorschriften für die Bereitung von Flechtenpräparaten mehr. Die Islandflechte wird jedoch als Droge noch in folgenden Pharmakopöen aufgeführt: *Ph. Austrica* 1906, *Ph. Belge* 1930, *Ph. Danica* 1933, *Ph. Estonica* 1937, *Codex Medicamentarius gallicus* 1937, *Ph. Helvetica* 1941, *Ph. Hungaria 1934*, *Ph. Italica* 1929, *Ph. Japonica* 1932 und

Ph. Nederlandica 1936 (14).

Der Gebrauch der Flechten als Arzneimittel ist jedoch nicht ganz verschwunden (26), besonders nicht bei den Heilkundigen aus dem Volke, sondern lebt immer noch als linderndes Mittel gegen Hustenreiz in ihren Arzneimischungen fort (6). Nach Untersuchungen über die Flechtensäuren kam der schon früher in der Hausindustrie üblich gewesene Gebrauch der Flechtensäuren zunächst als Farbstoffe (in erster Linie Saxatilsäure) von Neuem in Schwung (26).

Dessenungeachtet, dass in so vielen Pharmakopöen die Islandflechte, welche auch ein wenig Usninsäure enthält, erwähnt wird, so findet sie in der ärzlichen Praxis wenig Anwendung, denn als man anfing, die Droge in gereinigtem Zustand zu verwenden, d.h. als daraus oft die bitteren Stoffe, welche gerade wirksam waren, ausgelaugt wurden, büsste die Droge ihre Wirksamkeit ein, und sie wurde natürlich seltener oder gar nicht mehr gebraucht.

Die Untersuchungen über die antibiotische Wirkung der Flechten auf den *Staphylococcus aureus* und den *Bacillus subtilis* u.s.w. haben das Interesse an der Flechtenforschung wieder ins Leben gerufen (2). Die Folge davon war die Entdeckung von immer mehr antibiotisch wirkenden Flechten und Flechtensäuren. Die diesbezügliche Literatur ist bis jetzt noch nicht besonders umfangreich. Es ist festgestellt worden, dass das aus der *Buellia canescens*-Flechte isolierte Diploicin das Wachstum der Diphterie- und Tuberkelbazillen hemmt (1). Ein in vielen Flechten vorkommender, das Wachstum der Tuberkulosebakterien hemmender Stoff war die Usninsäure (8, 23), welche Säure schon im Jahre 1843 gefunden worden war (18). Ähnliche Wirkung wurde ausserdem bei der Vulpin-, d-Proto ichesterin-, Lichesterin-, Dihydrolichesterin-, Physod- und Diffrachtasäure festgestellt (8, 23). Viele Flechten hatten hemmende Wirkungen auf die Sarcina, den Staphylococcus, Streptococcus, B. megatherium und Corynebact. diphteriae sowie schwach auf den E. Coli und Proteus vulgaris (24, 25). Das kristallinische Derivat des Atranorins schien den *Proteus vulgaris* spezifisch zu beeinflussen, indem es sein Wachstum 1:10000 hemmte. Bei einigen Flechten wurde Wirkung auf die pathogenen Dermatophyten konstatiert (24).

Da die Flechten offenbar irgendeinen Stoff enthielten, welcher

auch auf das *Myobacterium tuberculosis* Einfluss hatte, nahmen wir
die als Volksarznei vorkommende Usninsäure zum Gegenstand
unserer Untersuchungen in Tierversuchen.

FRÜHERE UNTERSUCHUNGEN ÜBER DIE USNINSÄURE

Es ist bestätigt worden, dass die d- oder l-Usninsäure und deren
Natriumsalz das Wachstum mancher Mycobacterium-Arten sogar
als Verdünnung von 1:800.000 hemmend beeinflussen (23). Gleich-
zeitig wurde konstatiert, dass der aus der *Ramalina reticulata*-
Flechte isolierte Stoff C 16 H 14 O 6 hemmende Wirkung auf das
Wachstum verschiedener Tuberkulosestämme in Verdünnung von
1:20.000—1:50.000 hatte, und weiter, dass er bei subkutaner Ver-
abreichung den Verlauf der Tuberkulose bei Meerschweinchen
verlangsamte (8). Bei der Untersuchung eines antibiotischen Stoffes
der Flechte, welcher wie der oben erwähnte den Verlauf der Tuber-
kulose bei Meerschweinchen beträchtlich verlangsamte, wurde
festgestellt, dass derselbe mit der aus der *Cladonia silvatica* iso-
lierten d-Usninsäure identisch war (9).

Bei den weiteren Forschungen wurden manche Salze der Flech-
tensäuren mit Streptomycin und auch allerlei andere Usninsäure-
derivate untersucht (5, 7, 10, 11. 12, 13).

Wir haben die l-Usninsäure aus der Renntierflechte (*Cladonia
alpestris*) für den medikamentösen Gebrauch gewonnen (21).

Die erste Partie l-Usninsäure stellten wir aus trockener, zer-
stossener Renntierflechte her, indem wir sie mit kochendem Aceton
extrahierten, wobei der grösste Teil der Säure sich im Verlauf von
24 Stunden als nadelartige, hellgelbe Kristalle aus der Lösung
kristallisierte. Die so gewonnene Säure wurde durch nochmaliges
Kristallisieren gereinigt. Unsere Ausbeute aus der Droge war
1.5 %. Der Schmelzpunkt dieser nadelartigen Kristalle lag bei
202°C. (Der Schmelzpunkt von Marshaks Stoff war 191—192°C.)
Das spezifische Gewicht der Kristalle war 1.3. Die Säure und ihr
Natriumsalz wirkt auf der Zunge anfangs fast geschmacklos, aber
nach zwei Minuten schmeckt sie sehr bitter.

In konzentrierter Schwefelsäure löste die Usninsäure sich, und
wenn diese intensiv gelbe Lösung auf einem Uhrglas stehen ge-
lassen wurde, bildete sich auf ihrer Oberfläche nach einigen Stunden
ein schön blaues Häutchen, die darunter befindliche Flüssigkeit

war heller gelb. Diese Reaktion wurde durch die Feuchtigkeit der Luft beeinflusst, denn im Exicator hielt sich die in gleicher Weise behandelte Säure tagelang, ohne Häutchen zu bilden. Die gelbe Farbe der Usninsäure ist sehr intensiv, eine Glykoselösung von 1:3000 ist noch deutlich gelblich.

Aus der Droge lässt sich die Usninsäure auch durch Mikrosublimation isolieren, wobei die Säure sich erst als ölige Tropfen abscheidet, welche sich dann in gelbe Kristalle umwandeln.

Bei vermindertem Druck wurden die in den Abbildungen 1 und 2 gezeigten Kristalle erhalten.
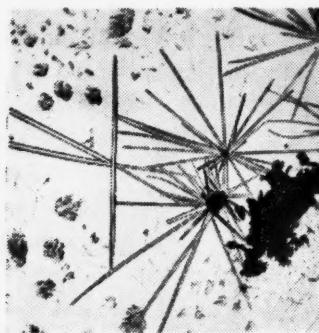
Abb. 1. Mikrosublimat der
l-Usninsäure.
Druck 8 mm 205°C. 36-fache
Vergrösserung.
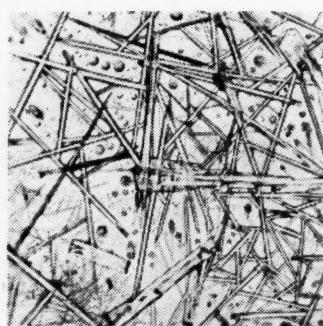
Abb. 2. Mikrosublimat der
l-Usninsäure.
Druck 5 mm 223°C. 36-fache
Vergrösserung.

Das Absorptionsvermögen der l-Usninsäure wurde in Chloroform (1,0166 mg/ml) untersucht. Die Flüssigkeitsschicht war 1 cm dick. Es wurden folgende Extinktionswerte [1] mit folgenden Lichtwellenlängen (μ) erhalten.

Einige Kristalle Usninsäure wurden in heissem Aethylen- oder Propylenglykol aufgelöst. Es wurde eine gelbe Lösung erhalten, unter welche ein wenig Alkohollösung von Paraphenylendiamin gemischt wurde. Das Gemisch färbte sich grün, und die Farbe blieb einige Stunden lang ûnverändert stark. Die Intensität der grünen Farbe war, wie festgestellt wurde, abhängig von der Konzentration

---

[1] Die Extinktionsbestimmungen der Usninsäure wurden im Biochemischen Forschungsinstitut mit dem Beckmann'schen Quarzpektralphotometer auf dem Gebiet der ultravioletten Strahlen und mit dem Pulfrich-Photometer auf dem Gebiet der sichtbaren Lichtstrahlen im pharmazeutischen Laboratorium der Universität ausgeführt.
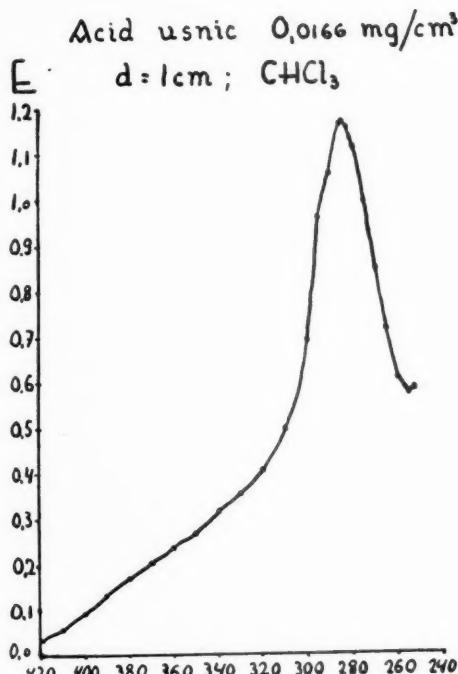
Kurven 1
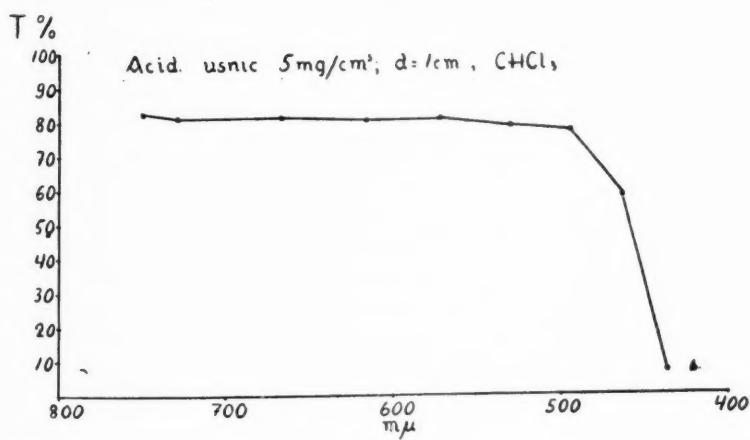
Kurven 2

der Usninsäurelösung, und noch bei Verdünnung von 1:4000 wurde diese Farbe wahrgenommen. Unter Anwendung von geeigneten Mengen Reagenz und Säure liess sich mit dem Kolorimeter von Dubosq die Konzentration der Usninsäurelösung bestimmen, wobei als Norm reine Usninsäurelösung von bekannter Konzentration benutzt wurde. Auf diese Weise konnte in stark verdünnten (1:1000, 1:4000) Lösungen die Konzentration der Säure bestimmt werden.

Die hemmende Wirkung der l-Usninsäure auf das *Mycobacterium hominis* wurde noch in den Verdünnung 1:50.000 beobachtet (24).

Die hemmende Wirkungen der l-Usninsäure auf die Tuberkelbazillen, welche deutlich in vitro beobachtet worden sind, erfordern weitere Klärung auch in Tierversuchen.

Da wir in einem orientierenden Versuch (4, 15, 20) Hinweise auf den möglichen Gebrauch der Usninsäure bei Menschen und Tieren gaben, setzten wir unsere Versuche fort, indem wir die Wirkung des Stoffes auf tuberkulotische Meerschweinchen ausprobierten.

EIGENE UNTERSUCHUNGEN

MATERIAL UND METHODE

In den Vorversuchen hatten wir einige Versuche gemacht, um die obere Grenze der Dosierung, ca 50 mg/die per os, zu finden.

Das eigentliche Versuchsmaterial bildeten 36 Meerschweinchen, welche möglichst gleichalt waren. Die Tiere wurden zuerst gewogen, wonach einem Teil der Meerschweinchen intraperitoneal 1/1000 mg Tb-Bazillen Suspension injiziert wurde. Bei den Versuchen wurden drei Stämme, humanen Typus, verwendet, nämlich Nr. 967, ein von einem gutartigen, produktiven Fall isolierter Stamm, Nr. 968, ein von einem produktiv-exsudativen Fall isolierter Stamm, sowie Nr. IV, ein von einem exsudativ malignen Fall isolierter Stamm. Die Dosierung der l-Usninsäure und die Anzahl der Versuchstiere gehen aus der Tabelle 1 hervor. Verabreicht wurde die Usninsäure per os mit einer Spritze, deren lange Nadel mit Kugelspitze versehen war. Alle Meerschweinchen wurden nach 8 Wochen getötet und obduziert. Die verschiedenen Organe wurden dann mikroskopisch untersucht. Es fiel auf, dass die mit Usninsäure behandelten Versuchstiere während der ganzen Dauer

TABELLE 1

ANZAHL DER VERSUCHSTIERE UND IHRE VERTEILUNG IN DEN VERSUCHEN

Versuchstiere und ihre Behandlung	Tb-Stamm			Meer- schweinchen ohne Tuberkulose	Zusammen
	267	268	IV		
Meerschweinchen ohne U-Säure [1]	4	4	5	3	16
» 15 mg U-Säure per os pro die	4	4	2	5	15
» 50 mg U-Säure per os pro die	0	0	2		2
» 5 mg U-Säure subkut. pro die	0	0	1	2	3
Meerschweinchen zusammen ..	8	8	11	9	36

[1] l-Usninsäure

des Versuchs guten Appetit hatten, insbesondere während der ersten 7 Tage.

ERGEBNISSE

MAKROSKOPISCHE UNTERSUCHUNG

Vor dem Töten der Meerschweinchen wurden dieselben ge-wogen, und die Gewichtsschwankungen wurden bei der Beurtei-lung der endgültigen Resultate berücksichtigt. Die Ergebnisse gehen aus der Tabelle 2 hervor.

Aus der Tabelle 2 ist ersichtlich, dass sich nichts Wesentliches über das Steigen oder Sinken des Gewichts der Meerschweinchen sagen lässt. Eine gewisse Gewichtsabnahme ist in der Gruppe, welche 50 mg Usninsäure per os pro die per Versuchstier erhalten hat, wahrzunehmen.

AUTOPSIE

Bei der makroskopischen Inspektion wurden in erster Linie die Milz, auch deren Gewicht, die Lungen, die regionären Lymph-drüsen und die Leber berücksichtigt. Auf Grund des Ausmasses der pathologisch-anatomischen Veränderungen wurde versucht, makroskop sch das Krankheitsbild zu beurteilen, wobei als Skala der Beurteilung die Bezeichnungen »schwer», mittelschwer», »sehr leicht» und »null» benutzt wurden. Die Resultate sind aus der Ta-belle 3 ersichtlich.

TABELLE 2

MAKROSKOPISCHER BEFUND

EINFLUSS DER l-USNINSÄURE AUF DAS GEWICHT VON NORMALEN UND TUBER-
KULOTISCHEN MEERSCHWEINCHEN

Gruppe	Intraperitoneale Dosis der Tuberkelbazillen 1/1000 mg	Nr. des Versuchstiers	Gewichtsschwankungen in Gramm während der Observationszeit
I	Tuberkelbazillus Nr. 967+	1	− 90
	U-Säure 15 mg per os/	2	+ 10
	pro die	3	− 10
		4	− 10
II	Tuberkelbazillus Nr. 968+	5	+ 110
	U-Säure 15 mg per os/	6	+ 390
	pro die	7	+ 230
		8	+ 185
III	Tuberkelbazillus Nr. IV — U-Säure 15 mg per os/ pro die	9	− 110
		10	+ 190
	U-Säure 50 mg per os/ pro die	11	− 230
		12	+ 30
	U-Säure 5 mg in Aethylen-glukol subkutan pro die	13	+ 90
		14	+ 60
I	Tuberkelbazillus Nr. 967	15	+ 10
		16	− 80
		17	− 10
		18	± 0
II	Tuberkelbazillus Nr. 968	19	+ 200
		20	+ 190
		21	+ 80
		22	+ 145
III	Tuberkelbazillus Nr. IV	23	+ 80
		24	+ 10
		25	+ 20
		26	+ 100
		27	+ 50
IV	Ohne Bazillen	28	+ 110
	U-Säure 15 mg per os/	29	+ 140
	pro die	30	− 65
		31	+ 70
		32	+ 220
V	Ohne Bazillen 5 mg subkutan	33	+ 180
VI	Ohne Bazillen	34	+ 205
		35	+ 135
		36	+ 110

TABELLE 3

MAKROSKOPISCHER AUTOPSIEBEFUND 56 TAGE NACH DER INFEKTIERUNG GETÖTET

Nr. des Versuchs-tieres	Gewichts-schwankun-gen in Prozent	Veränderungen			Gewicht der Milz in Gramm	Auswertung der pathologischen Veränderungen
		Lunge	Leber	Milz		
1	− 13	SL	0	M	1160	M
2	+ 1	0	0	0	910	SL
3	− 10	0	0	0	930	SL
4	− 2	0	0	SL	1190	SL
5	+ 18	0	0	M	1150	SL
6	+ 98	0	0	M−S	1410	M
7	+ 62	0	0	M	1000	SL
8	+ 54	0	0	SL	1060	SL
9	− 25	SL	S	S	378	M
10	+ 48	SL	SL	M	1610	SL
11	− 36	0	SL	SL	710	SL
12	+ 4	SL	0	SL	1000	SL
13	+ 19	SL	SL	S	1780	SL
14	+ 13	SL	M	S	1120	M
15	+ 1	0	0	SL	950	0
16	− 11	0	0	M	1210	M
17	− 1	0	0	S	1110	M
18	± 0	0	0	S	1110	M
19	+ 44	0	0	S	1650	M
20	+ 70	0	SL	M	1630	M
21	+ 13	0	0	S	1160	M
22	+ 24	0	0	S	1150	M
23	+ 21	SL	SL	M	1780	M
24	+ 3	S	S	S	3850	S
25	+ 4	M	S	S	3710	S
26	+ 25	S	S	S	1800	S
27	+ 11	SL	M	S	1750	M
28	+ 30	0	0	0	680	0
29	+ 38	0	0	0	1030	0
30	− 9	0	0	0	590	0
31	+ 9	0	0	0	760	0
32	+ 110	0	0	0	960	0
33	+ 90	0	0	0	680	0
34	+ 55	0	0	0	1000	0
35	+ 37	0	0	0	700	0
36	+ 28	0	0	0	700	0

1) O = Null   SL = sehr leicht   M = Mittel   S = Schwer
M-S = Veränderung zwischen Mittel und Schwer usw.

TABELLE 4
GEWICHT DER MILZ

Nr. des Versuchs-tiers	Tb-Stamm 1/1000 mg intraperitoneal	Durchschnitt-liches Gewicht der Milz	Mittelwert der durchschnittl. Gewichte der Milz
Infektierte, nicht behandelte Meer-schweinchen			
15, 16, 17, 18	967	1100	
19, 20, 21, 22	968	1400	1700
23, 24, 25, 26, 27	IV	2580	
Nicht infizierte Meerschweinchen			
28, 29, 30, 31, 32	Keine Bakterien. 15 mg per os pro die U-Säure	800	742
33	5 mg subkutan pro die U-Säure	680	770
34, 35, 36	Ohne Bakterien und ohne U-Säure	800	800
Infektierte und be-handelte Meer-schweinchen			
1, 2, 3, 4	15 mg u-Säure per os pro die, Stamm 967	1047.5	
5, 6, 7, 8	15 mg —»—, 968	1155.0	
9, 10	15 » —»—, IV	999	1111.5
11, 12	50 » —»—, IV	855.0	
13, 14	5 » subkutan, IV	1500.0	

Besonders fallen die Gewichtsschwankungen der Milz ins
Auge, welche am deutlichsten aus der Tabelle 4 hervorgehen.
Wenn man als Gruppe der gesunden Tiere sowohl die mit Usnin-
säure behandelten Meerschweinchen als auch die eigentlichen
Kontrollen d.h. die nicht behandelten nicht infizierten Tiere be-
trachtet, so erhält man als deren Mittelgewicht der Milz 742 mg
resp. 800 mg, Mittelwert 770 mg, während das durchschnittliche
Gewicht der Milz der nicht behandelten tuberkulotischen Tiere
1,700 mg betrug. Das Gewicht der Milz war also bei den infek-
tierten Meerschweinchen über zweimal so gross wie bei den ge-
sunden. Bei den mit Usninsäure behandelten, tuberkulotischen

Meerschweinchen betrug das durchschnittliche Gewicht der Milz 1,111 mg, also niedriger als bei den Versuchstieren ohne Usninsäure, aber trotzdem noch recht hoch.

Das Krankheitsbild wies abhängig von dem Tb.-Stamm Unterschiede auf insofern, als bei den mit dem Stamm IV infizierten Meerschweinchen die Tuberkulose makroskopisch schwerer war als be den mit dem Stamm 967 infizierten.

MIKROSKOPISCHER BEFUND [1]

Die Resultate der mikroskopischen Untersuchung sind aus der Tabelle 5 ersichtlich.

Bei der Beurteilung wurden als Tuberkulose Veränderungen gerechnet, welche zwar nicht typisch sind, aber doch Tuberkulose sein können. Solche wurden als »sehr leichte» Fälle berücksichtigt.

Aus der Tabelle 5 ersieht man, dass die mikroskopischen Unterschiede zwischen den verschiedenen Gruppen keineswegs beträchtlich sind. Aus der Tabelle 6, in welcher die makroskopischen und die mikroskopischen Resul ate miteinander verglichen werden, geht hervor, dass bei dem von einem produktiven Falle isolierten Stamm zwischen den behandelten und den unbehandelten Gruppen kein Unterschied zu bestehen scheint. Die deutlichsten Unterschiede treten bei dem von einem exsudativen Falle isolierten Stamm auf, wo in der Gruppe der mit Usninsäure behandelten Versuchstiere nur halb so viel Nekrosen angetroffen werden wie bei den unbehandelten und desgleichen entsprechend mehr Bindegewebe.

Die Ausdehnung des Krankheitsprozesses und seine qualitative Natur sind zwei verschiedene Dinge. Zum Beispiel bei Nr. 9, wo die Veränderungen »sehr schwer» sind, ist die qualitative Natur der Krankheit als gutartig zu betrachten, weil die Verkalkungstendenz beträchtlich ist; der durchgeführte Kalknachweis nach Kossa zeigt in diesen Fällen deutliche Verkalkungstendenz. Auch in den übrigen Fällen, bei welchen der Kalknachweis durchgeführt wurde, zeigte sich bei den mit Usninsäure behandelten deutlich die gleiche Tendenz (19).

---

[1] Der mikroskopische Befund wurde kontrolliert von Dr. med. VILJO RITAMA, wofür wir ihm unseren besten Dank aussprechen.

N:o	1) Milz			2) Lunge		
	Tuberkel	Ver-käsung	Bindegewebe	Tuberkel	Ver-käsung	Bindegewebe
1	+++	++	++++	++	0	++
2	0	0	++	0	0	++
3	0	0	++	0	0	++
4	0	0	++	0	0	++
5	0	0	++	0	0	++
6	++++	++	+++	+	0	++++
7[1]	++	+	+++	0	0	++
8	+	0	+++	0	0	++
9	++++	+++	+	0	0	+++
10	0	++	+++	0	0	++
11[2]	0	0	++	0	0	+++
12	0	0	++	0	0	++
13[3]	++++	±	++	+	+	+
14	0	±	+	+	+	±
15	+	0	++	0	0	++
16	+++	0	++++	+++	++	+++
17	+	0	++	0	0	+++
18	+++	0	+	0	0	0
19	++++	±[5]	++++	0	0	++
20	++	±	+	++++	0	+
21	+	±	+	0	0	+
22[6]	+	0[7]	+	0	0	+
23	++++	+++	++	++++	+++[8]	+
24	++++	++++	+	++++	+++	+
25	+++	+	+	++++	+	++
26	++++	+	+	++++	+	++
27	++++	+	++++	+	0	++
28	0	0	++	0	0	++
29	0	0	++	0	0	++
30	0	0	++	+	0	++
31	0	0	++	0	0	++
32	0	0	++	0	0	++
33	0	0	++	0	0	++
34	0	0	++	0	0	++
35	0	0	++	0	0	++
36	0	0	++	0	0	++

Veränderungen.

O = null   ++++ = hochgradig   +++ = mittelmässig

3) Leber			4) Bauchspeicheldrüse			Bemerkungen
Tuberkel	Binde-gewebe	Ver-käsung	Tuberkel	Ver-käsung	Binde-gewebe	
+	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
+	0	+ +	0	0	+ +	
+ + +	+ + +	+ + +	+ +	0	+ +	[1] Lymphdrüse + + + +
0	0	+ +	0	0	+ +	+ + + + + +
0	±	+ +	0	0	+ +	
0	0	+ + + +	0	0	+ +	
0	+ + + +	+ +	0	0	+ +	[2] Lymphdrüse 0 0 0
0	0	+ +	0	0	+ +	
0	0	+ + + +	0	0	+ +	[3] Lymphdrüse + + + + +
+	0	+ +	0	±	+ +	[4] Nekrosen
0	0	+ +	0	0	+ +	
+ + +	+ +	+ + +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	[5] Nekrose
+	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	[6] Lymphdrüse
0	0	+ +	0	0	+ +	± 0 0
						[7] Nekrose
+	0	+ +	0	+	+ +	[8] Lymphdrüse
+ + +	+	+ +	+	+	+ +	+ + + +
+ + + +	+ + +	+ +	0	0	+ +	+ + + + + +
[9]						[9] Lymphdrüse
+ + + +	+ + +	+ +	+ + +	+ + +	+ +	+ + + + + + + +
+ + + +	+ + + +	+ +	+ + + +	+ +	+ +	+ +
0	±	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	
0	0	+ +	0	0	+ +	

+ + = gering   + = vereinzelt   ± = unbestimmt

BESPRECHUNG DER ERGEBNISSE

Tabellen 6 und 7 stellen eine Zusammenfassung der Untersuchungen dar.

Die Dosierung 15 mg pro Versuchstier per os scheint vorteilhafter zu sein als 50 mg. Bei beiden Meerschweinchen, welche Usninsäure subkutan erhalten hatten, waren die durch die Krankheit verursachten Veränderungen umfangreich (schwer).

Die durch den von einem exsudativen Falle iso ierten Stamme hervorgerufenen Veränderungen waren schwerer als diejenigen, welche der von einem produktiven Falle isol erte Stamm verursacht hatte. Sowohl in den behandelten als auch in den unbehandelten Fällen waren die makroskopischen Veränderungen gross. Bei den behandelten Versuchstieren waren wen ger, aber grössere Tuberkel gebildet worden als bei den unbehandelten.

Das mikroskopische Bild war verschieden. In der Gruppe der mit Usninsäure behandelten Versuchstiere ist das Bindegewebe im Vergleich zu den unbehandelten deutlich vermehrt. Bei dem mit Stamm IV durchgeführten Versuch fallen besonders die Veränderungen in der Leber auf, welche in der Gruppe der mit Usninsäure behandelten Tiere verhältnismässig gross sind. Die Menge des sich mit Hämatoxylin basophil färbenden Zellgewebes inmitten der fast nekrotischen Gebiete zeigte sich auch überall als deutliche Tendenz zu Verkalkung (Kalknachweis positiv), was Verlangsamung des Prozesses bedeuten dürfte.

Die Bedeutung der verschiedenen Stämme für die entstandene Meerschweinchen-Tuberkulose sieht man ausser an dem Ausmass der Veränderungen vor allem in deren Beschaffenhe t, was mit früheren Untersuchungen übereinstimmt (16).

ZUSAMMENFASSUNG

1) Makroskopisch weichen die mit l-Usninsäure behandelten Meerschweinchen von den unbehandelten etwas ab. Dies zeigte sich am deutlichsten in der Milz, deren Gewicht sowie in der Anzahl und Grösse der Tuberkel.

2) Mikroskopisch war in der Gruppe der mit l-Usninsäure behandelten Versuchstiere auch in den malignen Fällen die Kalknachweis (nach Kossa) positiv und das Bindegewebe vermehrt.

TABELLE 6

VERGLEICH DER MAKROSKOPISCHEN UND MIKROSKOPISCHEN RESULTATE BEI
DEN MIT l-USNINSÄURE BEHANDELTEN TUBERKULOTISCHEN MEERSCHWEINCHEN

Nr. des Versuchstiers	Milz		Lunge		Leber		Bauchspeicheldrüse		Andere Organe
	Autopsiebefund	Histologisch. Befund	Autopsiebefund	Histologisch. Befund	Autopsiebefund	Histologisch. Befund	Autopsiebefund	Histologisch. Befund	
1	M	SL	SL	SL	0	SL	0	0	
2	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	
4	SL	0	0	0	0	0	0	0	
5	M	0	0	0	0	0	0	0	
6	S	M−S	0	M	0	SL	0	0	
7	M	M S	0	0	0	M	0	SL	7/Lymph-
8	SL	SL	0	0	0	0	0	0	drüse S
9	S	sehr S	SL	0	S	SL	0	0	
10	M	M	SL	0	SL	0	0	0	
11	SL	0	0	0	SL	0	0	0	
12	SL	0	SL	0	0	0	0	0	
13	S	M	SL	SL	SL	0	0	0	
14	S	SL	SL	SL	M	SL	0	0	
15	0	SL M	0	0	0	0	0	0	
16	M	M	0	SL	M	M	0	0	[1] Ähnlich wie
17	S	S[1]	0	0	0	0	0	0	Epithelzell-
18	S	S−M	0	0	0	0	0	0	gewebe
19	S	M	0	0	0	0	0	0	
20	M	S−M	0	M	SL	SL	0	0	20/Nekrose
21	S	M	0	0	0	0	0	0	22/Lymph-
22	S	SL	0	0	0	0	0	0	drüse SL
23	M	M−S	SL	M−S	SL	SL	0	SL	23/Nekrose
24	S	sehr S	S	S	S	S	0	SL	23/Lymph-
25	S	M	M	M	S	SL	0	M	drüse S
26	S	M	S	M	S	S	0	M	26/Lymph-
27	S	M	SL	SL	M	S	0	M	drüse S
28	0	0	0	0	0	0	0	0	
29	0	0	0	0	0	0	0	0	
30	0	0	0	SL	0	0	0	0	
31	0	0	0	0	0	0	0	0	
32	0	0	0	0	0	0	0	0	
33	0	0	0	0	0	0	0	0	
34	0	0	0	0	0	0	0	0	
35	0	0	0	0	0	0	0	0	
36	0	0	0	0	0	0	0	0	

TABELLE 7

ZUSAMMENFASSUNG SOWOHL ÜBER DIE MAKROSKOPISCHEN ALS AUCH DIE MIKROSKOPISCHEN VERÄNDERUNGEN BEI DEN MIT l-USNINSÄURE BEHANDELTEN TUBERKULOTISCHEN MEERSCHWEINCHEN

Nr. des Meer-schweinchens	Gewichts-verände-rungen in Prozent	Autopsie-befund	Histologi-scher Befund	Auswertung der pathologi-schen Ver-änderungen der Milz	Bemerkungen
1.	− 13	M	SL	M	
2	+ 1	0	0	0	Kalknachweis nicht
3	− 10	0	0	0	ausgeführt
4	− 2	VS	0	SL	
5	+ 18	M	0	SL	
6	+ 98	S	M−S	S	»
7	+ 62	M	M	M	
8	+ 54	SL	SL	SL	
9	− 25	S	sehr S	S	Kalknachweis
10	+ 48	M	M	M	positiv
11	− 36	SL	0	SL	»
12	+ 4	SL	0	SL	
13	+ 19	S	M	S	»
14	+ 13	S	SL	M	
15	+ 1	0	SL	SL	Kalknachweis nicht
16	− 11	M	M	M	ausgeführt
17	− 1	S	0	M	
18	± 0	S	S−M	S	
19	+ 44	S	M	M	
20	+ 70	M	S−M	M	»
21	+ 13	S	M	M	
22	+ 24	S	0	M	
23	+ 21	M	M−S	S	
24	+ 3	S	sehr S	S	Kalknachweis
25	+ 4	S	M	M	negativ
26	+ 25	S	M	M	
27	+ 11	S	M	M	
28	+ 30	0	0	0	
29	+ 38	0	0	0	Kalknachweis nicht
30	− 9	0	0	0	ausgeführt
31	+ 9	0	0	0	
32	+ 110	0	0	0	Kalknachweis negativ
33	+ 90	0	0	0	Kalknachweis nicht ausgeführt
34	+ 55	0	0	0	Kalknachweis
35	+ 37	0	0	0	nicht ausgeführt
36	+ 28	0	0	0	

3) Die Anzahl der Nekrosen war bei den mit l-Usninsäure behandelten Tieren etwas kleiner.

4) Die Unterschiede zwischen den mit l-Usninsäure behandelten und den unbehandelten Tieren waren am deutlichsten bei denjenigen, welche mit einem von einem exsudativen Falle isolierten Stamm infektiert worden waren. Bei von produktiven Fällen isolierten Stämmen waren die Unterschiede nicht so klar.

LITERATURVERZEICHNIS

1. BARRY, V. C.: Nature (Brit.), 1946:*158:*13.
2. BURKHOLDER, P. R., und EVANS, A. W.: Bull. Torrey Bot. Club. 1945:*72:*157.
3. BUSTINZA, F., und LOPEZ, A. C.: An. Jardin Bot. Madrid 1948:*7:*1.
4. HEILALA, P., und SIINTOLA, S.: Farm. Aikak. 1949:*11:*199, 203.
5. KLOSA, J. K.: Pharm. Zentralhalle für Deutschland 1949:*6:*165.
6. La Rousse Medical, Paris 1924.
7. MARSHAK, A., und al.: Proc. Soc. Exp. Biol. 1949:*70:*565.
8. MARSHAK, A.: Public Health Reports, 1947:*62:*3.
9. MARSHAK, A., BARRY, G. T., und CRAIG, L. C.: Science 1947:*106:*394.
10. MARSHAK, A., und HARTING, JANE: J. of Cellular and Comparative Physiology 1948:*3:*321.
11. MARSHAK, A., und KUSCHNER, M.: Public Health Reports 950:*65:*131.
12. MARSHAK, A.: J. of Cellular aud Comparative Physiology 1950. In Press Cit. MARSHAK (11).
13. NELSON, L.: The Biological Bulletin 1948:*95:*286.
14. Pharmacopea Austrica, 1906.
    »   Batava, 1805.
    »   Belge, 1930.
    »   Danica, 1933.
    »   Estonica, 1937.
    »   Fennica, I, 1819, V, 1915.
    »   Hungaria, 1934.
    »   Italica, 1929.
    »   Nederlandica, 1936.
    »   Universalis, Weimar 1846.
    Codex Medicamentarius gallicus, 1937.
15. PÄTIÄLÄ, R.: Ann. Med. Exper. Biol. Fenn. 1949:*27:*151.
16. PÄTIÄLÄ, J.: A. Tub. Scand. 1948:*3:*227.
17. PÄTIÄLÄ, R., PÄTIÄLÄ, J., SIINTOLA, S., und HEILALA, P.: Suomen Kemistilehti, 1948:*21:*127.
18. a) ROCHLEDER und HELDT: Liebigs Annalen der Chemie, 1843:*48:*1.
    b) KNOP, W.: J. f. Pract. Chemie 1844:*36:*122.
19. ROMEIS, B.: Taschenbuch der mikroskopischen Technik, München 1942. S. 547.

20. SIINTOLA, S.: Farm. Aikak. 1949:*9*:1.
21. SIINTOLA, S., HEILALA, P., PÄTIÄLÄ, J. und PÄTIÄLÄ, R.: Suomen Kemistilehti 1948:*21*:179.
22. SHIBATA, S., UKITA, T., TAMURA, T. und MIURA, V.: Japanese Med. J. 1948:*152*.
23. STOLL, A., BRACK, A., und RENZ, J.: Experimentia, 1947:*3*:3.
24. VARTIA, K. O.: Ann. med. Exper. Biol. Fenn. 1949:*27*:46.
25. VARTIA, K. O.: Ann. med. Exp. Biol. Fenn. 1950:*28*:7.
26. ZOPF, W.: Die Flechtenstoffe, Jena 1907.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF
HELSINKI

en

J.

STUDIES ON PHAGUS LACERANS

(STREPTOCOCCIC BACTERIOPHAGE B)

By

M. E. PARMALA

In spite of the great number and frequency of Streptococcus strains, no more than 7 separate strains of phages affecting them have been found (3, 14). It is true that Grumbach (16), in studying stored cultures, states having observed in approx. 60 per cent of the colonies »nibbled edges», which he ascribes to phage action. However, he did not isolate the phages, and it is not known whether several phage strains or some other cause accounts for the deformity of the colonies. Streptophages have been isolated from the blood of septicaemia patients (23), from spontaneous infection in rabbits (4), and by the ordinary method from sewage (9, 14, 17). Wasiliew (26) in East Europe has isolated a strain which, because of its weak action and the rapid formation of a resistant strain, is probably, however, identical with Evans's strain E.

It must possibly be ascribed to the infrequency of streptococcic phages that very few studies have been made of them, and the greatest part of the extensive material of phage studies deals with coli, dysentery and staphylococcal phages, easier to find and to handle. Streptococcic phages have been most thoroughly studied by Evans (8, 9, 10, 11, 12, 13), Evans & Sockrider (14) and Evans & Verder (15). As these studies, however, deal mainly with the classification of streptococci with the aid of phages, the author

has considered it justifiable to study certain properties of strepto-coccic phages considering the results arrived at with other phages, and their effect in statu nascendi on available streptococcal strains.

The phage used in the experiments, *Phagus lacerans* (3) or streptococcic phage B, and the corresponding sensitive strain 563, were obtained from Dr. Alice Evans about 10 years ago. The phage has been kept in broth in a refrigerator, and the bacterium dried from blood broth in vacuum also in refrigerator over the same period.

METHODS

In studying the effect in statu nascendi, Danish Griffith's type strains, certain North American strains resistant to phage action, and some domestic, recently isolated strains as well as their variants, and two resistant variants of sensitive strains were used.

The tubes used in the experiments had 2 ml of broth, and when larger quantities were needed, 10 ml. The culture medium was of the following content:

Bacto-Tryptose	20.0
Yeast Extract »Difco»	5.0
NaCl	8.0
Glucose	0.5
Buffer solution	22.0
H_2O	1000.0

pH 7.4—7.6

Buffer solution:

KH_2PO_4	1.45
Na_2HPO_4, 2 H_2O	7.6
Aq. dest. .... ad	100.0

To blood broth and blood agar 10 per cent of defibrinated horse blood was added. Active serum agar contained 10 per cent of fresh horse serum. Inactive serum agar contained 10 per cent of horse serum inactivated for half an hour at 56° C. The agar medium contained 1.2 per cent Agar Difco.

The quantitative study of phage action was carried out mainly in a tube series with ten-fold dilutions, which, according to several investigators, yields more accurate results than plague count by spreading on an agar plate (6, 22) (the result being expressed as the inverse of exponent value $= e_L$). In certain cases a plate experiment has been used to count the number of phage particles; 0.05 ml phage dilution was spread by glass rod on an even, strong bacterial inoculum, dried for half an hour at 37° C, and the ensuing plaques were counted after 24 hours incubation.

RESULTS WITH PHAGE FILTRATE

Both the sensitive strain and the phage had stood the long preservation well. In diluting straight from the storage tube the titre value obtained was e_L 5, while the plaque test showed distinct plaques. After one passage the titre reached its maximum value e_L 9 in the dilution series, and e_L 7 in the plaque test; nor were any higher values attained by continued propagations.

When 0.05 ml phage dilution, e_L4 and e_L6, was evenly spread on heavily inoculated agar plate (1 drop of 12 hour culture), distinctly discernible differentiated plaques become visible after 4 hours incubation, and later they grow in size. On plates containing active serum the plaques, after 24 hours, are 2 mm in diameter, on inactive serum agar plates 2.5—3 mm, on plain culture agar after e_L4 inoculation plaques grow together and individual plaques cannot be distinguished, and after e_L6 inoculation the diameter of plaques is 3—3.5 mm. Hence the serum is found to have a distinctly preventive influence on the growth of plaques. With dilution e_L6 the plaques average 45 in number. The number of phages in the original undiluted tube is therefore approx. 900 million/ml. On plates cultivated simultaneously in an atmosphere containing CO_2 (3 per cent) no essential difference is observed. The improved growth of the bacterium in CO_2 thus does not affect the size of the plaques to the same extent as does the serum content of the culture medium. After 24 hours of cultivation a thin translucent membrane of resistent bacteria is found on the plaques of every plate.

OBSERVATIONS ON BACTERIAL GROWTH AND PHAGE PROPAGATION

Seven ten-tube series were inoculated with 16-hour bacterial culture diluted to 10^{-5} 0.05 ml, the bacterial number in each tube averaging 20 in ml, and phage was added immediately to the first series so that the final dilution values in the tubes became $e_L 1 - e_L 9$; the last tube remained as bacterium control, from which 0.05 ml was taken for spreading on the blood agar plate for the bacterial count. All the series were taken to an incubator (37° C). Series II was similarly treated with phage after an hour, and 0.05 ml were taken from the control tube to blood plate for bacterial count. In the same way, phages were added to the other series after 2, 4, 6, 7, and 8 hours, and bacterial count taken from control tube on blood plate. From the control tube of the last series the bacterial count was taken as late as after 10 hours. The first slight turbidity was observed after 8 hours in all tubes of the last series, and in the last few tubes of the first series. After 9 hours, turbidity was observed towards the last tubes of each series, while the first tubes of the last series began to clear up. After 10 hours turbidity had increased in the middle series, while the first tubes of the last two series continued to become clear. After 11—13 hours, all series clearing up; continued up to 24—30 hours. The growth of the first resistant strain was observed as a slight turbidity after 24 hours. Maximum titre was attained after 30 hours. Maximum titres were $e_L 9$ in series IV—VI, corresponding to a bacterial growth of 4, 6 and 7 hours before phage addition. The ninth tube of Series VI, however, rapidly became turbid again, and thus a more permanent maximum titre was obtained with phage additions after 4 and 6 hours growth.

Distinct regularity in the development of the resistant strain was observed in 24-hour cultures (Table I, in brackets), even though the occurrence of resistant strain deviating from this rule was observed subsequently in individual tubes. In the regular development of the resistant strain the proportion phage — bacteria was found to be largely the same, with due allowance for errors (Series I tube 8 approx. 18: 40, Series III tube 7 approx. 18: 15, Series IV tube 6 approx. 18: 40, and Series V tube 5 18: 65). The possible errors are due to the chain shape of streptococci, which in counting the colonies gives one colony with several bacteria,

TABLE I

PHAGE INFLUENCE ON BACTERIAL CULTURES OF VARIOUS AGES, WITH A SMALL
INITIAL BACTERIAL COUNT, 20/ML

Series	Inocul. Time, Hours	Bact. Count at Phage Addition	Phage Dilutions 10^P									Bact. Control
			−1	−2	−3	−4	−5	−6	−7	−8	−9	
I	0	20	—	2	—	—	—	—	2	(4)	• 2	○ 4
II	1	25	2	—	2	—	2	2	2	—	• 4	4
III	2	75	—	—	—	—	—	—	(4)	3	• 4	4
IV	4	2000	2	—	—	2	2	(4)	—	4	2	• 4
V	6	32,500	—	—	—	—	(4)	—	—	4	2	• 4
VI	7	> 250,000	4	—	—	—	—	—	—	2	• 2	○ 4
VII	8	> 250,000	2	—	—	—	—	—	3	—	• 4	4

— = completely clear 1—4 = different degrees of turbidity
• = maximum titre completely cleared
○ = maximum titre partially cleared
(3) = regular resistant strains

and to the uneven suspension of phage particles in phage suspension.
The quantity of bacteria in the tubes at the different times of
phage addition is illustrated by the bacterial growth curve (Fig. 1),
drawn on the basis of agar plate cultures. The phage quantity,
undiluted, was, in the above plaque test, found to be 900 million/ml.

A second series, in which the number of tubes per series was
12, the initial bacterial count approx. 1,750/ml, with phage addition
after 0, 2, 4, 6, 8 and 10 hours, was made, to check the results
of the previous series. Turbidity was first observed after 7 hours
in the last series, and turbidity and clearing up took place mainly
in the same way as in the previous experiment. Maximum titre,
however, was reached after 12 hours, $e_L 9$ full clearing and $e_L 10$
slight clearing in series III and IV, corresponding to 4 and 6 hours
of bacterial growth before phage addition. The first resistant
growth was observed after 20 hours in one tube (6/II), while re-
sistant strain in other tubes developed only after 24—48 hours.

Similar regularity in the growth of resistant strain was observed
in this series too (Table II, in brackets), with few exceptions,
although the development, in regard to time, was not equally
regular. When heavier bacterial growth was involved the counting
was still more uncertain than in the previous experiment. Sufficient

TABLE II

PHAGE INFLUENCE ON BACTERIAL CULTURES OF VARYING AGES, WITH MODERATE INITIAL BACTERIAL COUNT, 1750/ML

Series	Inocul. Time, Hours	Bact. Count at Phage Addition	Phage Dilutions 10^P											Bact. Control
			−1	−2	−3	−4	−5	−6	−7	−8	−9	−10	−11	
I	0	1,750	—	—	3	—	—	—	—	—	• 4	4	4	4
II	2	5,600	—	2	—	—	—	(3)	—	—	• 4	4	4	4
III	4	130,000	—	—	—	(3)	(2)	—	—	—	—	• 4	o 4	4
IV	6	4.4 mill.	—	—	(4)	—	—	—	—	—	—	• 4	o 4	4
V	8	100 mill.	—	(2)	—	—	—	3	1	1	• 4	o 4	4	4
VI	10	700 mill.	—	—	—	—	3	2	—	—	• 4	4	4	4
VII	12	750 mill.	—	3	2	2	2	—	2	• 2	4	4	4	4

  — = completely clear   1—4 = different degrees of turbidity
  • = maximum titre completely cleared
  o = maximum titre partially cleared
(3) = regular resistant strains

uniformity, however, was observed in the phage — bacterium ratio to prove the results of the previous experiment. Further it was found that the resistant strains deviating from the rule were formed, more distinctly still, at a certain distance from the regular formation. Numbers under »Bact. count at phage addition» in Table II correspond to the different hours of phage addition, according to colony count, on the growth curve (Fig. 1). The amount of phages used in the experiment undiluted was approx. 13.5 million/ml.

PHAGE INFLUENCE ON OTHER STRAINS

The recently isolated domestic strain TS (from acute tonsillitis) was found sensitive to filtrate with a maximum titre of $e_L 5$. In spite of several passages the titre could not be brought any higher, *nor did propagation with this »foreign» strain cause any reduction of titre tested with the original sensitive strain 563.* Strains marked M_1, M_2, S_1, S_2 and S_3 were found fairly sensitive to B/563 phage filtrate. The propagation of the phage failed, however, with these strains. And phage growth could not either be observed in experiments where the filtration was omitted, as tubes to which $e_L 1$ phage-bacterium mixture from a cleared up tube was added to fresh growth in the proportion of 1: 10, turned completely turbid.

No adaptation was thus observed with this phage. Of the 58 diffe-
rent bacterial strains studied, the filtrate had a strong effect on
16 per cent, clearing completely several tubes, either in strong or
weak concentration; a fair effect on 14 per cent, clearing completely
a couple of tubes and preventing the growth partially in a couple
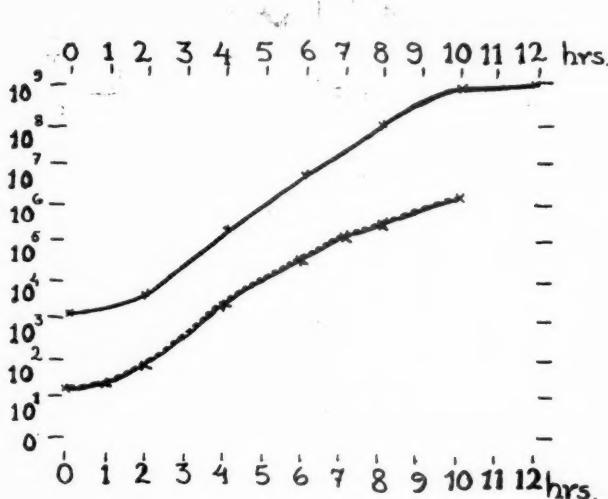
Fig. 1. — Bacterial growth curve, worked out on the basis of colony count,
bact./ml.

of tubes, either in strong or weak concentration; a slight effect
on 10 per cent, resulting in partial prevention of growth in some
tubes, either in strong or weak concentration. The balance (60 per
cent) were totally resistant to direct filtrate influence.

INFLUENCE OF QUANTITATIVE PROPORTIONS IN STATU NASCENDI

The influence in statu nascendi was studied in greater detail
with strain H6. To series of five tubes was added each of the three
factors in declining concentrations, e_L 1, 3, 5, 7, and the last
without the factor in question, so that all dilutions with the different
factors were present in 125 tubes. The two bacteria were pipetted
into the tubes first, and then the phage, each in fixed dilution.
Fig. 2 gives 26-hour readings and possible later clearing.

Phage action "in statu nascendi"
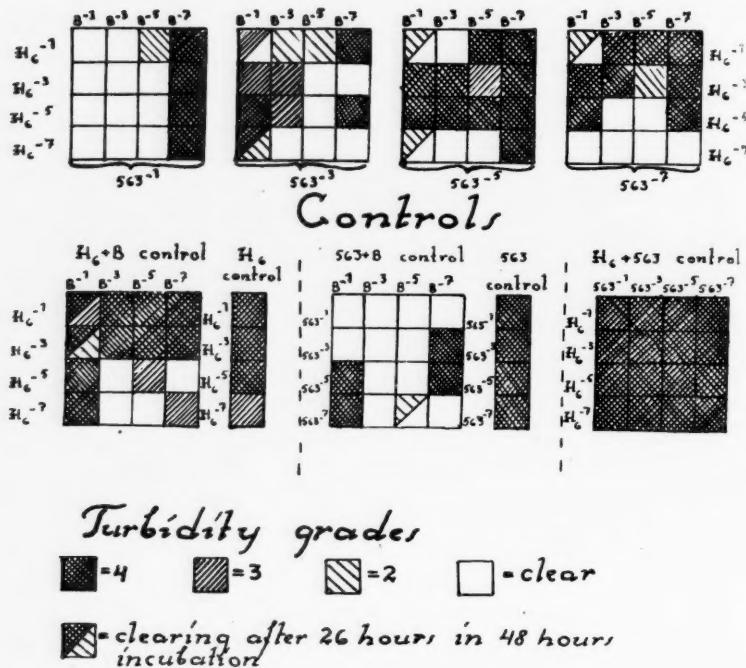
Fig. 2. — Phage influence in statu nascendi with different dilution values of strains 563 and H6 and of phage B (26-hour reading).

563	undiluted	approx.	12.5 million/ml.
H6	»	»	1.8 »
Phage	»	»	13.5 »

According to colony count, bacterial quantity, undiluted, of sensitive strain 563 is approx. 12.5 million/ml, and of the foreign strain H6 1.8 million/ml. Undiluted phage quantity was approx. 13.5 million/ml. The results show that best result is not always achieved by using an abundance of phage, and a great phage quantity alone affects a great quantity of foreign bacteria in a partially clearing manner. The same proportion, with smaller phage and bacterial quantities, does not result in full clearing, and thus the effect is not dependent solely on the proportions in which the

different factors are added. With the quantity of sensitive strain declining, the optimum proportion of foreign bacteria-phage approaches the proportion in which phage filtrate alone affects the foreign strain.

EFFECT IN STATU NASCENDI ON DIFFERENT STRAINS

In investigating the effect in statu nascendi in larger series and as routine work, separate additions of both phage and the sensitive strain are, however, inconvenient and involve a great deal of work. Therefore a mixture of sensitive strain 563 10^{-2} (4-hour culture) and phage in dilution 10^{-2} was prepared; both of them, at the moment of mixing, cooled to $0°$ C, and stirred for 30 mins. at $0°$ C. Two sets of series were prepared with declining quantities of the mixture and of different quantities of foreign bacterial strain H6. Due to the different bacterial quantities in each test the results of the series, with small quantities of mixture, did not correspond to each other. With a mixture quantity of 0.1 ml, and 0.1 ml or less of foreign bacterium, 4-hour culture, complete solution, however, was achieved in both tests. In spite of the uncertainty of results, effect in statu nascendi on other bacterial strains was also studied, by adding 0.1 ml of mixture to the 4-hour culture dilution. The first tube contained 0.1 ml of foreign culture and mixture, the second 0.05 ml of foreign culture and 0.1 ml of mixture, and the third 0.05 ml of foreign culture alone — to serve as growth control. The results arrived at were: among the 58 strains studied, complete solution entered or growth was completely prevented in 48 per cent, partial solution or prevention of growth was observed in 17 per cent, while in 34 per cent no effect compared with the control tube was discernible. In different experiments with the same foreign strain the effect was observed either immediately or after 48 hours, which is probably due to the chance quantities of foreign bacterium employed in the experiments (bacterial quantity varied prior to dilution). In four cases no effect in statu nascendi was obtained, although distinct solution was observed with mere filtrate, and the reverse took place in 20 cases, among them e.g. the two resistant varieties of the sensitive strain, isolated in connection with previous tests.

DISCUSSION

It is found that phages may be kept in refrigerator in the form of a filtrate of broth culture for quite long periods without losing their effectivity. The decline of titre from e_L9 to e_L5, according to Lominski et al. (20), may be considered as due to spontaneous agglutination, and no real destruction will take place in these conditions even over a period of ten years. A decline of titre of corresponding extent has previously been observed by Lominski (19), with staphylococcal, Shiga, coli- and subtilis phages, in four years. Applebaum et al. (1) attained in one year, with staphylo-coccal and coli-phage, a reduction of 1 titre degree in a refrigerator at $+9°$. Although this result does not fully correspond to the values obtained by Lominski (19), with a reduction in two weeks, at $+4°$, of $1-4$ titre degrees, it does not exclude the possibility of spontaneous agglutination, bearing in mind that different phage strains were used in the experiments.

The preventive effect of normal serum in culture medium on other phages has been observed by several researchers (2, 24, 5). On finding staphylococcal phages on which serum has no preventive effect, Rakieten assumes the preventive effect to be due to anti-phage substances present in the serum. According to Colvin (5), however, the effect is not specific, due to the preventive effect of serum on the propagation of phages, but not on adsorption or solution. The present results are in favour of this assumption as, if the effect were dependent on anti-phage substances, no plaques could be expected in complete absence of adsorption, while the preventive effect on the propagation of phages comprehensibly reduces plaque growth. That no mere bacterial growth-promoting effect of the serum is in question is indicated by the fact that plaques, formed in an atmosphere containing CO_2, did not essentially differ from those grown in ordinary atmosphere, although CO_2 markedly promotes the growth of streptococci.

As in the plaque test, a positive result is readable as soon as within $4-4.5$ hours, while the tube test requires at least 10 hours before a reliable result can be obtained, the plaque test is to be preferred if the results must be available early. As the maximum titre value is encountered in 4 and 6 hour cultures in the tube test,

with bacterial growth in logarithmic phase, it is generally advisable to use 4-hour cultures for the inoculation of phage dilutions.

Papers on the formation of a resistant strain with other phages have previously been published (25, 21). According to Wartio-vaara (25), a resistant strain of *Salmonella Breslau* is developed most rapidly with fairly abundant bacterial quantity under the influence of a large phage quantity. Luria et al. (21), again, indicate that a resistant strain of coli-phages originated solely in bacterial mutation, with no assistance from phage. The present investigations, however, tend to show that phage also plays, without doubt, a certain part in the origination of a resistant strain, as the early formation of resistant strain in tubes in which the proportion phage-bacteri a is approximately the same is hardly a mere accident, several tubes and two experiments yielding the same result. In addition, a postponed formation of resistant strain is observed in tubes with a small bacterial amount and large phage amount, or vice versa. In the latter, the opportunities for mutation, with large bacterial quantity, are good. In the former, provided phage action, according to Doubly et al. (7), primarily increases bacterial metabolism, which results in a more rapid entering of turbidity, the multiplication brings about good prospects of an entering of mutation. It is true that Krueger (18) maintains that no stimulating effect is discernible but that bacterial growth, after phage addition, is equal in extent to that prior to addition. However, in the course of the present investigations the author has encountered cases in which the tubes containing phages became turbid much more quickly and markedly than the control tubes before the entering of lysis. However, suspecting a possible technical error, the author paid no serious attention to the matter until, in studying the effect in statu nascendi, results were observed to support Doubly's opinion. E.g. the apparent resistance observed in statu nascendi with four strains, although a distinct clearing or even complete solution with filtrate is found, may be ascribed to the stimulating effect of phage. In statu nascendi the phages are adsorbed in part to the sensitive strain (563), in part to the »foreign» strain, ostensibly to a much lesser extent to the latter. Slight adsorption in the foreign strains results in livelier metabolism and, hence, more rapid multiplication, so considerable that the lytic effects of phage are suppressed. Also in the experiment shown in Fig. 2 the

addition of phage surplus, without lytic effect, observed by Evans (13) as well, who states that solution takes place either with concentrated or weak phage dilution both on filtrate and in statu nascendi, may perhaps be ascribed to the primarily stimulating effect of phage.

Phagus lacerans reveals no tendency to adaptation, and even after numerous passages maximum titre remains at the original figure characteristic of each different strain, and the effect on the original sensitive strain does not change in propagation with some less sensitive strains. With still less sensitive strains the propagation is not successful, although distinct effect upon them can be observed with phage filtrate of the sensitive strain.

SUMMARY

1. It was found that phage stands storage well. A phage suspension in broth culture filtrate kept in a refrigerator for 10 years was active in a 10^{-5} dilution.

2. The preventive effect of normal horse serum in culture medium on plaque growth but not on their formation, was ascertained, and may be due to the reduction of multiplication of phages.

3. Effect of phage additions of different amounts in the various phases of the streptococcal growth curve was followed in detail, as well as the development of resistant secondary growth.

4. It was found that a given proportion of phage-bacteria promotes the origination of resistant strain, which, hence, is not dependent on occasional mutation alone.

5. Of the 58 different streptococcal strains studied, the filtrate had a strong influence on 16 per cent, fair on 14 per cent and weak on 10 per cent. The balance (60 per cent) resistant. Phagus lacerans did not display any tendency to adaptation in these experiments, nor did propagation with a less sensitive Finnish strain cause any reduction of titre tested with the original sensitive American strain 563.

6. The best results in the study of effect in statu nascendi were found if the relative concentration of the sensitive strain was not too small. Phage had to be used in several different dilutions.

7. Of the 58 strains studied, the effect in statu nascendi proved to be strong on 48 per cent, weak on 17 per cent and nil on 34 per cent. In 4 cases the effect in statu nascendi was nil, although the filtrate made a distinct effect on these strains, and in 20 cases vice versa.

8. It was found, sometimes, that phage primarily stimulated bacterial growth, estimated as a rapid rise in turbidity before the subsequent clearing up of the culture.

REFERENCES

1. APPLEBAUM, M., and FRISBEE, F. C.: J. Lab. Clin. Med. 1939:*24:*1290.
2. APPLEBAUM, M., and MACNEAL, W. J.: J. Inf. Dis. 1931:*49:*225.
3. BERGEY's Manual of Determinative Bacteriology. Williams & Wilkins. Sixth edition, 1948.
4. CLARK and CLARK: Proc. Soc. Exp. Biol. Med. 1927:*24:*635.
5. COLVIN, M. G.: J. Inf. Dis. 1932:*51:*527.
6. DOERR, R., and GRÜNINGER, W.: Zschr. f. Hyg. 1922:*97:*209.
7. DOUBLY, J., and BROMFENBRENNER, J.: Proc. Soc. Exp. Biol. Med. 1933:*30:*732.
8. EVANS, A. C.: J. Bact. 1934:*27:*49.
9. EVANS, A. C.: Science 1934:*80:*40.
10. EVANS, A. C.: J. Bact. 1936:*31:*423.
11. EVANS, A. C.: Ibid 1940:*39:*597.
12. EVANS, A. C.: Ibid 1940:*40:*215.
13. EVANS, A. C.: Ibid 1942:*44:*207.
14. EVANS, A. C., and SOCKRIDER, E. M.: Ibid 1942:*44:*211.
15. EVANS, A. C., and VERDER, E.: Ibid 1938:*36:*133.
16. GRUMBACH, A.: Zentralbl. f. Bakt. I Orig. 1930:*118:*206.
17. KENDRICK, P., and HOLLON, H. C.: J. Bact. 1931:*21:*49.
18. KRUEGER, A. P., and NORTHROP, J. H.: J. Gen Phys. 1930:*14:*223.
19. LOMINSKI, I.: Compt. rend. Soc. de Biol. 1939:*130:*1086.
20. LOMINSKI, I., and KRASSNOFF, D.: Ibid 1938:*129:*1081.
21. LURIA, S. E., and DELBRÜCK, M.: Genetics 1943:*28:*491.
22. NICOLLE, P.: Compt. rend. Soc. de Biol. 1941:*135:*548.
23. PIARKOWSKI, G.: Med Klin. 1922:p.474.
24. RAKIETEN, M. L.: J. Immunol. 1933:*25:*127.
25. WARTIOVAARA, T. W.: Zentralbl. f. Bakt. I Orig. 1939:*144:*512.
26. WASSILIEW, A.: Mikrobiol. J. 1938:*5:*3 Ref. Zentralbl. f. Bakt. Ref. 1940:*136:*479.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

RESISTANCE OF E. COLI TO AUREOMYCIN IN VITRO

By

W. J. KAIPAINEN

Experiments were carried out in 1948 by Price, Randall and Welch (2) to find out if the resistance of bacteria to aureomycin can be increased *in vitro*. In titrating the resistance of their strains to aureomycin the bacteria were transferred from the first test tube showing growth to the following dilution series. The changes seen after fourteen transfers were as follows (Table 1):

TABLE 1

INCREASE OF RESISTANCE TO AUREOMYCIN

Micro-Organism	Initial Sensitivity μg per ml,	After 14 Transfers μg per ml.	Increase
B. subtilis	0.04	2.5	62
Sal. typhosa	1.2	5.0	4.1
E.coli...............	0.6	10.0	16.6
Kl. pneumoniae	2.5	80.0	32.0
Pr. vulgaris	1.2	640.0	533
Sar. lutea	0.15	0.3	2
S. aureus	0.08	0.08	0
St. hemolyticus	0.15	10.0	66.6

When studying the increased resistance of E. coli to streptomycin, Price, Randall, Chandler and Reedy in 1947 (1) found the following changes in three different strains of E. coli (Table 2):

TABLE 2

INCREASE OF RESISTANCE TO STREPTOMYCIN IN VITRO

Resistance of E.coli str. 1 increased 428—fold after 14 transfers

| » | » | » | str. 2 | » | 32—fold | » | » | » |
| » | » | » | str. 3 | » | 100—fold | » | » | » |

According to the above table the increase in the resistance of E. coli strains to streptomycin varies considerably, but seems to be greater than the increase in resistance to aureomycin (Table 1).

Personal Investigations

It was the object of the study reported here to carry out certain experiments on the change of resistance of E. coli to aureomycin in vitro. The following points were investigated.

1. How rapid is the increase in the resistance of E. coli *in vitro* when the aureomycin concentration is not high enough to inhibit the growth of the bacterium?

2. Is resistance increased also when the aureomycin concentration is already bacteriostatic?

3. Is the increased resistance a characteristic which readily disappears when no further doses of aureomycin are given?

Technique

Nutrient broth pH 7.0 was used as culture medium. A dilution of 10^{-4} was made from a 24-hour broth culture of E. coli and 1 ml was drawn with a pipette into each test tube of the series. One ml of aureomycin with a concentration of 1 mg per ml was then added into the first test tube and carefully mixed, after which 1 ml of the mixture was transferred to the following test tube. This procedure was continued until each test tube contained 1 ml of the mixture and the concentration of aureomycin always decreased by onehalf compared with the preceding test tube. The test tubes were closed with a cotton wool plug and placed in an incubator ($+ 37°$ C). Growth readings were taken after 24 hours. Bacteria were now taken from the first turbid test tube for the next series and a dilution of 10^{-4} was made into broth and cultures were made as on the preceding day. Using this technique 14 transfers were made during successive days.

Simultaneously with these series, control series were made to observe the change in the resistance of the same strain of E. coli when culturing it from day to day in broth without aureomycin (Tables 3 b and 4 b).

TABLE 3 a

INCREASE IN THE RESISTANCE OF E.COLI TO AUREOMYCIN IN VITRO

Strain 1

Test Tube No.	1	2	3	4	5	6	7	8	9	10	11
Concentration μg/ml	500	250	125	62.5	31.2	15.6	7.8	3.9	1.9	0.97	0.47
Serial No. of transfers in nutrient broth with aureomycin 1	−	−	−	−	−	−	−	−	−	+	+
—»— 2	−	−	−	−	−	−	−	+	+	+	+
—»— 3	−	−	−	−	−	−	+	+	+	+	+
—»— 4	−	−	−	−	−	−	+	+	+	+	+
—»— 5	−	−	−	−	−	−	+	+	+	+	+
—»— 6	−	−	−	−	−	+	+	+	+	+	+
—»— 7	−	−	−	−	(+)	+	+	+	+	+	+
—»— 8	−	−	−	−	−	+	+	+	+	+	+
—»— 9	−	−	−	−	−	+	+	+	+	+	+
—»— 10	−	−	−	−	−	+	+	+	+	+	+
—»— 11	−	−	−	−	−	+	+	+	+	+	+
—»— 12	−	−	−	−	−	+	+	+	+	+	+
—»— 13	−	−	−	−	−	+	+	+	+	+	+
—»— 14	−	−	−	−	−	+	+	+	+	+	+

Growth +, Slight Growth (+), No Growth −.

TABLE 3 b

RESISTANCE OF E.COLI IN CONTROL SERIES USING NO AUREOMYCIN

Strain 1

Test Tube No.		1 8		9	10	11
Concentration μg/ml		500	3.9	1.9	0.97	0.47
Serial No. of transfers in nutrient broth	1	−	−	−	−	+	+
» » » » » » »	2	−	−	−	+	+	+
» » » » » » »	3	−	−	−	+	+	+
» » » » » » »	4	−	−	(+)	+	+	+
» » » » » » »	5	−	−	−	+	+	+
» » » » » » »	6	−	−	(+)	+	+	+
» » » » » » »	7	−	−	−	+	+	+
» » » » » » »	8	−	−	−	+	+	+
» » » » » » »	9	−	−	−	+	+	+
» » » » » » »	10	−	−	−	+	+	+
» » » » » » »	11	−	−	−	+	+	+
» » » » » » »	12	−	−	−	+	+	+
» » » » » » »	13	−	−	−	+	+	+
» » » » » » »	14	−	−	−	+	+	+

The first strain used (Strain 2, Tables 3 a and 3 b) was a strain of E. coli which had been stored in the laboratory for six months. The fairly even increase in the resistance during the first six transfers (up to 16 fold) was striking (Table 3a, series 1—6). After this, no increase was observed. The resistance increased 4-fold after the first transfer (Table 3a). This might partly be due to the fact that the resistance was increased to some extent when the bacteria was released from the unfavourable storage conditions as indicated by the control series (Table 3 b, series 1—2).

E. coli str. 2 isolated from urine was used in the second experiment, (Tables 4 a and 4 b).

The increase in resistance took place after the first transfers also in this case and no change occurred after the fifth transfer. In the control series without aureomycin (Table 4 b) a slight gradual decrease was noticed in the resistance, possibly due to the fact that the sensitivity of the bacteria increased after removal from the urine.

TABLE 4 a

INCREASE IN THE RESISTANCE OF E.COLI TO AUREOMYCIN IN VITRO

Strain 2

Test Tube No.		1	2	3	4	5	6	7	8	9	10
Concentr. μg/ml		500	250	125	62.5	31.2	15.6	7.8	3.9	1.9	0.97
Serial No. of transfers in nutrient broth with aureomycin	1	—	—	—	—	—	—	—	+	+	+
—»—	2	—	—	—	—	—	—	+	+	+	+
—»—	3	—	—	—	—	—	—	+	+	+	+
—»—	4	—	—	—	—	—	+	+	+	+	+
—»—	5	—	—	—	—	+	+	+	+	+	+
—»—	6	—	—	—	—	+	+	+	+	+	+
—»—	7	—	—	—	—	+	+	+	+	+	+
—»—	8	—	—	—	—	+	+	+	+	+	+
—»—	9	—	—	—	—	+	+	+	+	+	+
—»—	10	—	—	—	—	+	+	+	+	+	+
—»—	11	—	—	—	—	+	+	+	+	+	+
—»—	12	—	—	—	—	+	+	+	+	+	+
—»—	13	—	—	—	—	+	+	+	+	+	+
—»—	14	—	—	—	—	+	+	+	+	+	+

The change in resistance caused by aureomycin was also studied with E. coli strain fresh by isolated from the stools, (Strain 3), but no increase in the resistance was observed.

W. J. KAIPAINEN

TABLE 4 b

RESISTANCE OF E.COLI IN CONTROL SERIES USING NO AUREOMYCINE

Strain 2

	1	...6	7	8	9	10
Test Tube No.	500	15.6	7.8	3.9	1.9	0.97
Concentr. µg/ml						
Serial No. of transfers in nutrient broth 1	−	−	−	+	+	+
» » » » » » » 2	−	−	−	+	+	+
» » » » » » » 3	−	−	−	+	+	+
» » » » » » » 4	−	−	−	+	+	+
» » » » » » » 5	−	−	−	+	+	+
» » » » » » » 6	−	−	−	+	+	+
» » » » » » » 7	−	−	−	+	+	+
» » » » » » » 8	−	−	−	+	+	+
» » » » » » » 9	−	−	−	(+)	+	+
» » » » » » » 10	−	−	−	(+)	+	+
» » » » » » » 11	−	−	−	(+)	+	+
» » » » » » » 12	−	−	−	−	+	+
» » » » » » » 13	−	−	−	−	+	+
» » » » » » » 14	−	−	−	−	+	+

In addition to the above tests the resistance was determined several times for bacterial populations which had grown only once in aureomycin of different concentrations (3.9, 1.9 and 0.97 µg/ml) which did not inhibit the growth. The original resistance of 7.8 micrograms (the growth-inhibiting amount of aureomycin per ml) increased in each test to 15.6 micrograms (the same amount as in Table 4 a, series 1—2). Thus the fact whether bacterial populations had grown in aureomycin concentrations 3.9, 1.9 or 0.97 µg/ml did not influence the increase in the resistance.

IS THE RESISTANCE INCREASED WHEN THE AUREOMYCIN CONCENTRATION IS ALREADY BACTERIOSTATIC?

When observing the changes in the resistance of E. coli Str. 2 a specimen was taken from the last clear test tube of the series (Table 4 b, test tube 7) by means of a loop and placed on a blood-dish. The resistance of the bacterial population was determined in the usual way. No increased resistance was observed. However, when a specimen was taken from the same test tube after 48 hours of growth when the contents of the test tube were turbid, the resistance had increased in the same proportion as in table 4 a, series 1—2. It thus appears probable that the growth of the coli in aureomycin solution is necessary for the increase of the resistance to aureomycin.

STABILITY OF THE INCREASED RESISTANCE

After fourteen transfers the E. coli strain obtained from urine (Strain 2) was cultured in broth by daily transplantations to fresh broth. The resistance was determined in the usual manner after 7, 14, 21 and 28 days. No diminishing of the increased resistance was noticed.

SUMMARY

1. The resistance of two E. coli strains to aureomycin in vitro increased during the first 5 to 6 transfers, when the aureomycin concentration was so weak that growth was not inhibited. No change was observed in the resistance of the third E. coli strain.

2. When the aureomycin concentration was bacteriostatic the resistance to aureomycin did not increase.

3. The increased resistance appeared to remain on the same level for four weeks by daily transfers in nutrient broth without aureomycin.

REFERENCES

1. PRICE, C. W., RANDALL, W. A., CHANDLER, V. L., and REEDY, R. J.: J. Bact. 1947: 53:481—488.
2. PRICE, C. W., RANDALL, W. A., and WELCH, H.: Ann. N. Y. Acad. Sci. 1948: 51 (2): 211—217.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

ANTIBIOTICS IN FINNISH HYMENOMYCETES

By

K. O. VARTIA

All the species collected and identified by N. Malmström of the Botanical Museum, University of Helsinki.

Since the discovery of penicillin a fairly extensive literature has accumulated on the subject of antibiotics in fungi. A large number of active species have been found in the genera of *Penicillium* and *Aspergillus* (21), whereas the *Fungi imperfecti, Phycomycetes* and *Ascomycetes* have proved relatively poor in this respect (21, 22). The higher fungi have become a subject of increasing interest in recent years.

In 1942—1943 Wilkins and Harris (23) studied 722 large species of *Basidiomycetes* and found that 70 of them exerted a strong action and 100 a weak action on *Stàphylococcus aureus* and/or *E. coli*. About 10 per cent of the 100 species of *Basidiomycetes* which were later studied by Wilkins and which were mainly destructive to wood also proved active against these bacteria (20). On the other hand, Robbins *et al.* (17) found about 200 active species in a corresponding American series comprising over 400 fungi. However, a different technique was employed in these tests and the investigators reported that a part of the inhibitory action was due to changes in the pH value. Mathieson (13) reported 40 active species of *Basidiomycetes* out of the 230 studied by them. Meyer (15), Rose (18) and Nemec (16) carried out tests on the action of different species of *Polyporus,* Melin et al. (14) on *Marasmius,* and Cova and Ratti (8) on *Boletus.* Working with *Cortinarius rotundiporus,* Atkinson (4) found the approximate inhibitory titre of an extract of this fungus against *Staphylococcus aureus* to be 1: 300,000.

Among the antibiotic substances isolated from the higher fungi, clitocybin extracted in 1945 by Hollande (10) from *Clitocybe candidus* was found to exert marked action against *Mycobact. tuberculosis*. In 1949 Hollande announced the clitocybin in crystals (11). Another active factor, nebularin, was found in *Clitocybe nebularis* by Ehrenberg, Löfgren, Hedström and Takman (9, 12). This substance differed from clitocybin in respect to thermostability and relative non-toxicity. They reported that the purest fraction was capable of inhibiting the growth of different TB strains in concentrations of from 1: 100,000 to 1: 1,000,000. The substance was ineffective against staphylococci, streptococci and the colon bacillus. Brian and McGowan (5) discovered from *Trichoderma viridis* a fungistatic substance which they called viridin. Arnstein, Cook, and Lacey *et al.* (2, 3, 6, 7) isolated from *Fusaria javanicum* a substance of the naphthoquinone type called javanisin and found that titres from 1: 50,000 to 1: 100,000 were bacteriostatic to tubercle bacilli. A crystalline substance which was extracted by Anchel *et al.* (1) from *Coprinus similis* and *Lentinus degeneris* was identified by them as 5-methoxy-p-toluquinone, and the minimum inhibitory titre for *Staphylococcus aureus* was found to range from 1: 100,000 to 1: 200,000.

Personal Investigations. — Preliminary tests were made with 118 species of *Basidiomycetes*, collected in the vicinity of Helsinki, against *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris*. Prior to the tests the fungi were stored in the refrigerator for about four weeks. In the case of *Staphylococcus aureus*, however, fresh fungi collected on the testing day were also used and where any difference was seen in the results both are given in Table 1, the first result referring to the fresh fungus.

The tests were carried out on dishes, using the technique earlier employed in tests with lichens (19). After 24-hours' culture a broth-bacteria emulsion was spread evenly on agar plates, the surface was allowed to dry, and pieces of fungi weighing on an average 10 mg and pounded with a 0.5 mm sterile puncher were placed on the surface. Readings were made after 18 hours and are listed in Table 1 as follows:

++ = inhibition zone over 5 mm in width, measured from
        the margin of the fungus;
+ = inhibition zone 2—5 mm wide;

TABLE 1

	Staph. aureus	Proteus vulgaris	E.coli	Wilkins and Harris	Mathieson	Robbins et al.
Amanita mappa Batsch.	0	0	0	0		
» muscaria (L.) Pers. ..	0	0	(+)	0	0	
» porphyria Alb. et. Schw.	0	0	0	0	.	
» rubescens Pers.	0	0	0	0		
Amanitopsis vaginata Bull. ..	0	0	0	0	0	
Armillaria mellea Vahl.	0	0	(+)	0		
Bjerkandera adusta (Willd.) Karst. = Leptoporus adustus (Willd.) Quél.	0	0	+			
Bjerkandera borealis (Fr.) Karst. = Spongipellis borealis (Wahl.) Pat.	0 +	0	+			
Boletus bovinus Fr.	0	0	0	0		
» elegans Schum.	0	0	0	0		
» luteus L.	0	0	+	0	0	
» rufus Schaeff. = versipellis Fr.............	0	0	(+)			
» scaber var. niveus Fr.	(+) +	0	+			
» scaber Bull.	0	0	+	0		
» variegatus Swarz.	0	0	0	0		
» viscidus L.	0	0	0	0		
Calodon aurantiacus (Batsch.) » Quél.	0 0+	0	+			
» cyathiformis (Schaeff.) Quél.	0	0	0			
» ferrugineus (Fr.) Quél.	0	0	+			
Cantharellus cibarius Fr.	+	0	0	S+	0	
» umbonatus Gmel.	+ 0			0		
Clavaria fistulosa Holmsk.	0	0	0			
» ligula Schaeff.	0	0	0			
Climacodon septentrionalis (Fr.) Karst.	0		0			
Clitocybe gilva (Pers.) Fr.	+	0	+	0		
» inversa (Scop.) Quél.	++	0	+	S++ C++		
» metachroa Fr.	++	+	0	S+		
» nebularis Fr.	0 0+	0	++(+)	0	0	
» odora (Bull.) Fr.	+	0	+	S++ C++		
» opaca Fr.	+	0	+			
Collybia butyracea (Bull.) Fr...	0 +	0	+	0		
» cirrhata Fr.	0	0	0			
» confluens Fr.	0	0	0			
Coprinus comatus (Schum.) Fr...	0	0	0	C+		
Cortinarius armillatus Fr.	+ 0	(+)	+	S+		
» camphoratus Fr. ..	0	0	0			
» cinnamoneus (L.) Fr.	0	0	0	0		
» pholideus Fr.	0	0	0			
» semisanguineus Fr.	(+)	0	(+)	0		

	Staph. aureus	Proteus vulgaris	E.coli	Wilkins and Harris	Mathieson	Robbins et al.
Cortinarius traganus Fr.	(+) +	0	+			
» torvus Fr.	0 +	(+)	+	0		
» triumphans Fr.	+ +	+	+	S+ C+		
» sp.	+	(+)	0			
Fomes igniarius (L.) Fr.	0	(+)	(+)	0		0
Fomitopsis pinicola (Swartz.) Karst. = Ungulina marginata (Fr.) Pat.	0	0	0			
Ganoderma applanatum (Pers.) Pat.	(+)	0	+	0	0	
Gomphidius glutinosus (Schaeff.) Fr.	0	0	0			
Hansenia pubescens (Schum.) Karst = Coriolus pubenscens Schum.	0 +	0	0			
Hansenia zonata (Fr.) Karst. = Coriolus zonatus (Fr.) Quél.	0	0	+			0
Hydnum (Sarcodon) imbricatum (Fr.) Quél.	0	(+)	+	S+		
» repandum L.	+ (+)	0	+	S+	S+	
Hygrophorus agathosmus Fr. ..	0	0	+			
» bicolor Karst. ..	0	0	+			
» conicus (Scop.) Karst.	0	(+)	0	0		
» hypothejus Fr. ..	0		0			
» pratensis (Pers.) Karst.	0	0	0			
Hypholoma capnoides Fr.	0	0	0	0		
» sublateritium (Schaeff.) Fr.	0	0	+	0	S+	S+
Inocybe geophylla (Bull.) Fr. ..	0	0	(+)	0		
» lacera Fr.	0	0	+	0		
Inonotus radiatus (Sow.) Karst. = Xanthochrous radiatus (Sow.) Pat.	0	0	+			0
Ischnoderma resinosum (Schrad.) Karst.	0	0	0			
Laccaria laccata (Scop.) Cook.	+	+	0	0	0	
Lactarius camphoratus Fr.	(+) 0	0	0	0		
» deliciosus (L.) Fr. ..	0	0	+	0	0	
» flexuosus Fr.	0	0	0			
» glyciosmus Fr. nec. Karst.	0	0	0	0		
» helvus Fr.	0	0	0			
» subdulcis (Bull.) Fr. sensu Karst.	0	0	0	0		
» torminosus (Schaeff.) Fr.	0	0	0	0		
» trivialis Fr.	0	0	+	0		
» turpis Fr.	+	0	+	S+		
» vietus Fr.	+	0	+	0		

	Staph. aureus	Proteus vulgaris	E.coli	Wilkins and Harris	Mathieson	Robbins et al.
Lenzites betulina (L.) Fr.	+	0	+	0		0
Lepiota acutesquamosa Weinm.	0	0	(+)	0		
» amianthina (Scop.) Fr.	0	0	+	0		
» clypeolaria (Bull.) Fr.	0	0	(+)			
» cristata (Alb. et Schw.) Fr.	0	0	0			
Marasmius perforans Fr.	+	+	+			
» oreades (Bolt.) Fr.	0 (+)	0	+	0	0	
» peronatus (Bolt.) Fr.	0	0	+	0		
» scorodonius Fr.	+	(+)	+*			
Mycena galericulata (Scop.) Karst.	0	0	+	S+		
» pura (Pers.) Fr.	0	0	0	0	0	
Omphalia umbellifera (L.) Fr.	(+) +			0		
Paxillus involutus (Batsch.) Fr.	0	0	(+)	0	0	S+
Phaeolus Schweinitzii (Fr.) Pat.	0	0	0	0		S+
Pholiota caperata Pers.	0 +	0	+			
» lucifera (Lasch.) Fr. ..	0	0	0	0		
» squarrosa Müll.	0	0	(+)	0		
Piptoporus betulinus (Bull.) Karst. Ungulina betulina (Bull.) Pat.	+	0	0	S+		S+
Polyporus frondosus Fr.	0	0	+			0
» sulphureus Fr.	0	0	+	S+		S+
Polystictus perennis (L) Fr. ..	0	0	+	0		
Psalliota arvensis Fr.	0	0	+	S++ C++	0	
» hortensis Cook.	0	0	+		0	
Russula aeruginea Fr.	0 (+)	0	0			
» decolorans Fr.	0	0	+			
» densifoli Secr.	0	(+)	0	0		
» emetica (Schaeff.) Fr.	+	0	+	0		
» foetens Pers.	0	0	0	0		
» fragilis Fr.	(+)	0	0	0		
» integra Fr.	0	0	+			
» lutea (Huds.) Fr.	0	0	0	0		
» paludosa Britz.	0	0	+			
» vesca Fr.	0	0	+	0		
Tricholoma album (Schaeff.) Fr.	(+)	0	+	0		
» equestere (L.) Fr.	0 0+	(+)	++	0		
» flavobrunneum Fr.	0	0	+			
» rutilans (Schaeff.) Fr.	0	0	+	S+ C+		
» virgatum Fr.	(+)	0	(+)	0		

* Melin et al S++.

($+$) = growth weaker around the fungus but no clear zone
seen;

0 = inhibition zone 0—1 mm.

The last three vertical columns show the results reported for
the same species by Wilkins and Harris, Mathieson, and Robbins
et. al. The bacterial organisms are designated by »S» for *Staphylo-
coccus aureus* and »C» for *E. coli.*

Discussion of the Results. — It was observed as a general feature
that the same species of fungus and even the same specimen fre-
quently gave diverging results when tested at different times. This
may provide an explanation for the fact that different investiga-
tors have obtained results which are not always in accord with
each other. In the present tests the cap and the stem, or the sur-
face and the marrow of a specimen were in some cases liable to give
divergent results. The activity of a fungus against bacteria some-
times seemed to become altered during refrigerator storage; in
most cases it appeared to increase. *Clitocybe nebularis* gave, without
apparent reason, contradictory results in the different tests.

A comparison of these results with those obtained by the same
technique in earlier tests with lichens (19) showed that the
action of the fungi was considerably more labile and of a different
general trend, as the lichen exerted a growth-inhibiting action
mainly on staphylococci (and other Gram-positive bacteria) but
only rarely on the colon bacillus, whereas the fungi appeared to
have a definite disposition to inhibit the growth of the latter.

The action of the fungi is classified in Table 2 according to the
different bacteria tested. Only those test results in which a clear
zone of inhibition was seen are included.

TABLE 2

Staph. aur. $+$ *E. coli* $-$	*Staph. aur.* $+$ *E. coli* $+$	*E. coli* $+$ *Staph. aur.* $-$	No inhibition	No. of species studied
8 (2 also *Proteus* $+$)	21 (2 also *Proteus* $+$)	30	55	111

SUMMARY

Tests were carried out with 111 species of *Hymenomycetes* by placing small pieces of fungi, pounded with a sterile puncher, on agar plates on which bacterial emulsion had been evenly spread. Growth-inhibiting action was clearly exerted on both *Staphylococcus aureus* and *Escherichia coli* by 21 species, on the coli alone by 30, and on the staphylococcus alone by 8. Four species of fungi appeared to inhibit the growth of *Proteus vulgaris*. The fungi appeared to have a disposition to inhibit the growth of the coli bacillus, contrary to what the case seems to be with lichens studied by the same technique.

REFERENCES

1. ANCHEL, M. et al.: Proc. Natl. Acad. Sci. U.S. 1948:*34*:498.
2. ARNSTEIN, H. R. V., COOK, A. H. and LACEY, M. S.: Brit. J. exp. Path. 1948:*27*:349.
3. ARNSTEIN, H. R. V., COOK, A. H. and LACEY, M. S.: Nature 1946 *157*:333.
4. ATKINSON, N.: Austral. J. exp. Biol. med. Sci. 1946:*24*:169.
5. BRIAN, P. W. and Mc GOWAN, J. C.: Nature 1945:*156*:144.
6. COOK, A. H. et al.: Nature 1947:*160*:31.
7. COOK, A. H. and LACEY, M. S.: Brit. J. exp. Path. 1945:*26*:404.
8. COVA, N. and RATTI, G.: Il Farmaco 1947:*2*:518.
9. EHRENBERG, L. et al.: Sv. Farm. Tid. 1946:*31*:645.
10. HOLLANDE, A. C.: Comt. rend. 1945:*221*:361.
11. HOLLANDE, A. C.: Comt. rend. 1949:*18*:7081.
12. LÖFGREN, N. et al.: Sv. Farm. Tid. 1949:*17*:321.
13. MATHIESON, J.: Austral. J. exp. Biol. med. Sci.: 1946:*24*:57.
14. MELIN, E., WILEN, T. and OBLOM, K.: Nature 1947:*159*:840.
15. MEYER, J. R.: Arq. Inst. Biol. Sao Paulo 1944:*15*:27.
16. NEMEC, P.: Chem. Zvesti 1947:*1*:169, 294.
17. ROBBINS, W. J. et al.: Bull. Torrey Bot. Cl. 1945:*72*:165.
18. ROSE, S. R.: Nature 1946:*31*:292.
19. VARTIA, K. O.: Ann. Med. exp. Biol. Fenn. 1949:*27*:46.
20. WILKINS, W. H.: Brit. J. exp. Path. 1946:*27*:140.
21. WILKINS, W. H. and HARRIS, G. C. M.: Brit. J. exp. Path. 1942:*23*:166.
22. WILKINS, W. H. and HARRIS, G. C. M.: idem 1943:*24*:141.
23. WILKINS, W. H. and HARRIS, G. C. M.: Ann. appl. Biol. 1944:*31*:261.

FROM THE DEPARTMENT OF PHYSIOLOGY, VETERINARY COLLEGE,
HELSINKI

THE EFFECTS OF BILATERAL VESICULECTOMY AND OF THE REMOVAL OF THE PROXIMAL LOBES OF THE PROSTATE ON THE FERTILITY OF RATS

By

REINO POHJOLA and GUSTAF ELFVING

The physiology of the seminal vesicles is still largely obscure, and the literature on the subject is scarce and old. Some studies have been published concerning the effects of the removal of these organs on the normal physiology of reproduction, but their results are highly conflicting. Thus, Steinach (10) and Walker (14) studied the effect of bilateral vesiculectomy in rats and found that copulation is not affected, but fertility is greatly lowered. Armistead (1) and Lawlah (8) found that this operation resulted in complete sterility and a decrease of sex activity in guinea-pigs, whereas Laurent (7) stated that the animals remain normal in both respects if only the vasa deferentia are not injured at the operation.

The effect of the removal of the proximal lobes of the prostate has been studied in rats only by Walker (12) and in guinea-pigs by Engle (3). Both state that sexual activity remains unchanged while fertility is reduced by about 50 to 60 per cent.

Iwanow (5) and some other investigators succeeded in producing pregnancy in rats and guinea-pigs through artificial insemination by taking sperm directly from the testes or epididymis and by diluting it with normal saline solution. Because of this the function of the accessory sex glands, chiefly the vesicles, has been considered to be only the dilution of the testis-epididymis secretion to facilitate its injection into the vagina.

The present investigation was carried out with the object of checking these results, which are highly conflicting as regards vesiculectomy and somewhat unexpected as regards proximal prostatectomy.

MATERIAL AND METHODS

The animal material consisted of 23 male rats of our own colony, 15 to 18 weeks old, tested and proved to be fertile immediately before the experiments, and of about 40 female rats which were also known to be fertile.

The distribution of the rats into different groups is shown below (Table 1):

TABLE 1

Group No.	Number of Rats	Organ Removed
1	8	None (the control group)
2	7	Vesicles and proximal lobes
3	4	Vesicles only
4	4	Proximal lobes only

All operations were performed under light ether anesthesia. The vesicles and the proximal lobes were exposed through a low central incision. In group 2 they were removed distal to a silk ligature tied at the root of the organs. In groups 3 and 4 the operation was continued: the membrane enveloping these organs was incised and they were carefully separated from each other. After ligation the vesicles (in group 3) or the proximal lobes (in the group 4) were removed. Extreme care was taken not to injure any of the surrounding structures, especially the vasa deferentia near the proximal part of the vesicles. (cf. Laurent).

Opportunity for mating was provided for the male rats on the 10th, 20th and 40th day after operation: they were placed in the same cage with a female rat in estrus. Vaginal smears were taken to establish whether copulation had occurred. The finding of a vaginal plug or of spermatozoa in the vagina was regarded as a sign of this. Afterwards the female rats were observed for pregnancy; they were isolated and a record was made of the placental sign and of an eventual litter.

RESULTS

The results are shown in table 2.

TABLE 2

Group No.	Days from Operation	Number of Rats	Vaginal Plug	Sperma-tozoa	Placental sign	Litter
1.	10	8	8	8	8	8
	20	8	8	8	8	8
	40	8	8	8	8	8
2.	10	7	0	3	1	1
	20	7	0	2	0	0
	40	7	0	1	0	0
3.	10	4	0	2	0	0
	20	4	0	1	0	0
	40	4	0	1	0	0
4.	10	4	4	4	4	4
	20	4	1	4	4	4
	40	4	0	4	4	4

The sexual behavior of the operated rats did not differ from the usual in any of the groups. All the experimental rats displayed an active interest in the females as soon as they were put in the same cage with them.

DISCUSSION

The results show that bilateral vesiculectomy and, of course, combined vesiculectomy and the removal of the proximal lobes of the prostate cause sterility and prevent the formation of the vaginal plug. In the group 2 there was one copulation resulting in pregnancy and in the birth of a litter but even this rat later proved to be sterile. This may have been due to some residual spermatozoa remaining in the urethra. That great care is necessary at the operations appears from one discarded case in group 2 in which fertility seemed to persist. However, the autopsy showed that the ligature was not quite at the root of the vesicle but that a small part of it and of the proximal lobes remained proximal to the ligature on both sides. Walker (14) reports a similar observation. But his results in rats submitted only to vesiculectomy (five rats of

twelve produced a litter) differ greatly from ours. However, he admits having noted at necropsy that in one of the rats there remained stumps full of secretion, but in the others the removal had been complete. In our series vesiculectomy immediately resulted in failure to produce the vaginal plug and in sterility.

The vaginal plug is a cylindric mass of coagulated semen adhering fairly firmly to the walls of the vagina. It forms a few minutes after copulation especially in rodents and falls out a few hours later. It was first observed by Leuckart (9) in the guinea-pig. Two layers have been recognised in it: the inner consists chiefly of coagulated vesicular secretion and mucus and contains a great number of spermatozoa, while the outer consists of cornified epithelial cells detached from the vagina and the uterus as a result of intense leukocyte infiltration (6, 11). The vesicular secretion coagulates in contact with the prostatic secretion. Camus & Cley (2) assumed that the latter contains a special enzyme »vesiculase», charasteristic to all rodents. Walker (13) found that it was produced only in the proximal lobes of the prostate and therefore applied to these the well-known term »coagulating glands». Engle confirmed this and asserted that the secretion of these two lobes is actually the only one that is capable of causing coagulation.

The significance of the vaginal plug has been much discussed. Leukart and other earlier investigators considered that its purpose was to prevent too early outflow of the semen. Lataste pointed out, in addition, that by functioning like the piston of a pump it favored the entrance of semen into the uterus. If what Hartman says of the oppossum is true in the case of rodents, i.e. that spermatozoa generally can reach the external os of the uterus only with the aid of simple peristalsis of the walls of the lateral vaginal canal, the vaginal plug could also be explained as an obstacle which the retrogressive waves in the walls of the lateral canal push towards the uterus (Engle's remark).

In our experiments the removal of the proximal lobes of the prostate left the rats fertile. In Walker's experiments 8 of 21 rats became sterile, and Engle reported that pregnancy resulted in 10 cases of 26. Armistead considered the decrease in fertility possibly due to an injury to the vasa deferentia at operation or to the absence of the vaginal plug.

An interesting finding in group 4 is that in all four cases the vaginal plug was formed even 10 days after the operation and in one case even 20 days after it. According to Engle, it should no longer form if the proximal lobes have been removed from the rat. True, Stockard & Papanicolaou state that vesicular secretion may sometimes coagulate even in the absence of prostatic secre-

tion, but it seems more probable that, in spite of extremely careful removal of the lobes, slight secretion from the stumps has continued for some time, and this has been sufficient to cause the formation of the plug.

In groups 2 and 3 spermatozoa were noted in the vaginal smears of only a few of the female rats, and the number of such cases seems to diminish as the time interval between the operation and the mating increases. Even with due regard for the reduction caused by vesiculectomy in the volume of the ejaculate, we feel justified in assuming that the matings were generally unsuccessful since in the other groups spermatozoa were always found.

SUMMARY

The investigation was undertaken for the purpose of finding out how the fertility of rats was affected by (1) bilateral vesiculectomy combined with the removal of the proximal lobes of the prostate, (2) bilateral vesiculectomy alone, and (3) the removal of the proximal lobes alone.

Bilateral vesiculectomy always resulted in a failure of the vaginal plug to form and in sterility. When only the proximal lobes of the prostate were removed, the fertility remained unchanged but the capacity to form the vaginal plug was lost. The plug is thus no prerequisite for fertile copulation.

REFERENCES

1. ARMISTEAD, R. B.: J. Exper. Zoöl. 1925:41:215.
2. CAMUS, L., & GLEY, E.: Compt. rend. Soc. biol. 1896.
3. ENGLE, E. T.: Anat. Rec. 1926:34:75.
4. HARTMAN, C. G.: Anat. Rec. 1924:27:293.
5. IWANOW, E.: Arch. f. mikrosk. Anat. 1911:77:240.
6. LATASTE, F.: Zool. Anz. 1883:6:115.
7. LAURENT, G.: Compt. rend. Soc. biol. 1931:106:47.
8. LAWLAH, J. W.: Anat. Rec. 1930:45:163.
9. LEUCKART, R.: Zur Morphologie und Anatomie der Geschlechtsorgane. Göttingen 1847. Quoted from STOECKEL & PAPANICOLAOU.
10. STEINACH, E.: Pfluegers Arch. f. Physiol. 1894:56:304.
11. STOCKARD, C. R., & PAPANICOLAOU, G. N.: Biol. Bull. 1919:37:222.
12. WALKER, G.: Johns Hopkins Hosp. Bull. 1901:12:77.
13. WALKER, G.: Johns Hopkins Hosp. Bull. 1910:21:182.
14. WALKER, G.: Johns Hopkins Hosp. Repts. 1911:16:223.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY
OF HELSINKI

HEALTHY CHILDREN AS CONTROL OF SEROLOGICAL REACTIONS IN SYPHILIS

By

R. PÄTIÄLÄ

The oldest test used in Finland for the serological diagnosis of syphilis is a modification of the WR (8), adopted as early as 1913. It is still employed in its original form and continues to meet high requirements for specificity (7). It is supplemented by the WR sensitized with cholesterol and by the Kahn reaction (6).

In working with serological tests for syphilis there constantly arises the question of their specificity, i.e. of the extent to which they show positive results in non-syphilitic cases. The findings made in the studies carried out in Finland in an effort to answer this question are shown in Table 1.

According to these investigators errors are present in the Wassermann reaction in 0.3 to 0.75 per cent and in the Kahn reaction in 0.5 to 1.5 per cent. The percentage of error varies in the different series, depending upon 1) the thoroughness of the clinical examination, 2) the criteria accepted for a clinical diagnosis of syphilis, 3) the number of syphilitics in the series, and 4) whether the false positive results include all errors or whether the technical errors have been eliminated.

If we examine the percentage of non-specific reactions in any series whatsoever from which the technical errors have been removed, the material which remains contains two components, i.e. 1) the biological errors and 2) the undiagnosed cases of syphilis. In

TABLE 1

EARLIER STUDIES IN FINLAND ON NON-SPECIFIC SYPHILIS REACTIONS

Investi-gator	Year of Study	Study Series	No. of Per-sons Exam-ined	Per cent of Sero-positive Reactions	Per cent of Non-specific Reactions	Technical Errors Eliminated from Non-specific Reactions
Streng Sievers Vuori	1933	Syphilitics Control serie	739 931	46.46	WR 0.5	no
Turunen	1935	Women's Clinic University of Helsinki	2390	Kahn and frequently WR 2.9	WR and Kahn 0.75	no
Honkanen	1936	Helsinki Tuber-culosis Hospital	611	Kahn only 5.2	Kahn 0.66	no
Sievers	1937	Syphilitics Control series	467 1640	6.45	WR 0.61	no
Maijala	1942	Medical Poly-slinic, Univer-city of Helsinki	10953	WR 2.54 Ch WR 2.78 Kahn 3.38	WR I 0.04 Ch WR 0.06 Kahn 0.11	no
Pikka-rainen	1943	Patients with chronic internal diseases (syphi-litics) control material	99 1987	4.75 WR 0.20 Kahn 0.30	WR 0.1 Kahn 0.5	no
Pentti-nen	1946	Women's clinic, University of Helsinki	18090	Kahn and frequently WR 2.49	WR 0.03 Kahn 0.5	Yes; biolog-ical error WR 0 % Kahn 0.1 %
Leineberg	1949	City of Vaasa	5684	WR 0.7 Kahn 1.27	0.19	no

order to obtain an idea of the part played by the error percentage caused by undiagnosed syphilis and thus be able to estimate the value of the biological error, I have studied a WR-Kahn series of children from 10 to 11 years old with whom the presence of acquired syphilis is excluded and only the possibility of undiagnosed con-genital syphilis remains.

THE WRITER'S INVESTIGATION

The blood specimens were collected from the elementary schools of 70 communes in different parts of Finland. The samples

were posted for examination. The total comprised 2,000 blood samples from children of a mean age of 10 to 11 years; both sexes were represented in approximately equal numbers. The children were, on the whole, healthy [1].

In cases in which the test gave a positive reaction one or more control tests were later made. The samples sent in from the rural districts were hemolytic in 122 cases [2]. The number of tested samples decreased therefore to 1,878 samples.

TECHNIQUE

The routine methods used in Finland were employed in this study (6).

RESULTS

The immediate results obtained in the study are shown in Table 2.

TABLE 2
IMMEDIATE RESULTS IN THE CHILD SERIES STUDIED

Result	No. of Samples
Positive	26
Negative	1,852
Hemolytic	122
	2,000

The reactions which were more or less positive were grouped as follows according to the degree of reaction:

WR +	WR +?	WR ±	WR −?			
3	1	3	1	=	Total	8
Ch WR +	Ch WR +?	Ch WR ±	Ch WR −?			
2	—	3	2	=	»	7
Kahn +	Kahn +?	Kahn ±	Kahn −?			
4	1	3	5	=	»	13

The above 28 reactions belong to 26 cases in each of which one reaction only was positive and to one case of congenital syphilis which gave positive reactions in all the tests.

---

[1] One sample from a case of congenital syphilis was intentionally included.

[2] The large number of rejected hemolytic samples is explained by the fact that the mailing distance was long and the work was carried out in winter. time (2).

TABLE 3

THE POSITIVE REACTIONS AND THEIR FIRST CONTROL TESTS

Reading	Earlier Reaction			First Control Test		
	WR	Ch WR	Kahn	WR	Ch WR	Kahn
+	3	2	4	1	1	3
+?	1	—	1	—	—	—
±	3	3	3	—	—	—
—?	1	2	5	—	—	2
—	0	0	0	—	6	8

The reactions were immediately controlled by means of a new blood sample. These findings are shown in Table 3.

The original reaction remained positive in the first control test in five cases only, i.e. one case was positive in all the reactions, two cases were Kahn positive, and two Kahn negative. The first was the case of congenital syphilis, in which the mother had been put under treatment. In one of the persisting Kahn positive cases the subject's familial history also indicated congenital syphilis. The other children (one Kahn positive and two Kahn negative?) were normal.

The last three cases were controlled once more and gave negative reactions.

If these three cases are regarded as biological errors and all those

TABLE 4

RESULTS OF SEROLOGICAL EXAMINATION OF 10—11 YEAR-OLD ELEMENTARY SCHOOL CHILDREN, GROUPED ACCORDING TO ANALYSIS OF THE NON-SPECIFIC REACTIONS

Serological Reaction Reading	Technical Error		Biological Error	
	No. of Cases	Per cent	No. of Cases	Per cent
WR +, +?, ±	6	0.32	0	
—?	1	0.05	0	
Total	7	0.37	0	0
Ch WR +, +?, ±	4	0.21	0	
—?	2	0.11	0	0
Total	6	0.32	0	0
Kahn +, +?, ±	6	0.32	1	0.05
—?	5	0.27	2	0.11
Total	11	0.59	3	0.16

which were negative in the second phase are considered technical errors, the following picture will be obtained of the series (Table 4).

In comparing this table with Table 1 we find that the high numbers for false positive results are primarily due to technical errors, but to some extents also to undiagnosed syphilis. This observation should not, however, be too much generalized, as the specificity of serological reactions is of a biogeographical nature. In some parts of the globe the biological error produced by certain diseases may be quite high.

SUMMARY

The writer has studied the so-called non-specific reactions in the serological tests used for diagnosing syphilis. The series studied consisted of 2,000 elementary school children in order to eliminate the errors caused by the wrong diagnosing of acquired syphilis. In 122 cases, or in 6.1 per cent, the samples were hemolytic. The 1,878 serologically-examined samples gave WR positive reactions in 8 cases, or in 0.43 per cent, Chol. WR positive in 7 cases, or in 0.37 per cent, and Kahn positive in 13 cases, or in 0.69 per cent.

A renewed examination, with fresh blood samples, showed that most of these results were technical errors. The percentage of biological errors was 0 per cent in the WR ad 0.05 per cent in the Kahn reaction. When comparing these results with earlier studies in Finland it would seem that the percentages of error estimated in them is excessively high, due chiefly to technical errors and undiagnosed cases of syphilis.

REFERENCES

1. HONKANEN, P.: Duodecim. Acta A. 1936:*19:*
2. KOULUMIES, ROLF: Ann. Med. Exp. Biol. Fenn. 1948:*26:*27.
3. LEINEBERG, O.: En Wasserman-massundersökning i Vasa. Vasa 1949.
4. MAIJALA, O.: Klinische Untersuchungen über die Heufigkeit und Art der seropositiven Spätlues in Finnland. Helsinki. 1942. Academic Thesis.
5. PENTTINEN, K.: On the Wassermann and Kahn Reactions during Pregnancy. Helsinki 1946. Academic Thesis.
6. PIKKARAINEN, J.: Duodecim 1943:*59:*212.

7. SIEVERS, O.: Acta Path. et Microbiol. Scand. 1937:*14*:427.

8. STRENG, Osv., and MURTO, J. A.: Duodecim 1913:*20*:178 and 257.

9. STRENG, Osv., SIEVERS, O., and VUORI, A. K.: Duodecim Acta A. 1943:*16*:1.

10. TURUNEN, A.: Duodecim 1935:*51*:831. Also in German.

SEROPOSITIVE SYPHILIS IN FINLAND 1939–1944 IN THE LIGHT OF BLOOD DONOR VOLUNTEER MATERIAL

By

TAUNO PUTKONEN and RISTO PÄTIÄLÄ

(Received for publication June 27, 1950)

In Finland as in the other countries involved in the war, the incidence of fresh syphilis recorded in official statistics increased considerably during and after World War II. Clinically established cases are, as a rule, placed under treatment and are entered in the statistics. On the other hand, little information is available on cases of previously unrecognized syphilis found by mass blood testing. Table 1 comprises the previous research in Finland on this subject.

Turunen (7), Maijala (3), Penttinen (5) and Leineberg (2) examined apparently healthy persons, Maijala's and Leineberg's cases consisting of factory workers and Turunen's and Penttinen's of pregnant women from Helsinki.

In a report on wartime blood donor volunteers Renkonen (6) mentions that in 1942 1.1 per cent of the over 18,000 persons examined gave positive seroreactions and in addition there were 1.5 per cent weak reactions, making a total of 2.6 per cent. He states that they were completely unaware of their condition and therefore would hardly have come under medical treatment had the blood test not been taken. Our object is to examine the entire wartime blood donor volunteer material in this respect. [1]

TABLE 1

PREVIOUS RESULTS OF MASS BLOOD TESTING FOR SYPHILIS IN FINLAND

Investigator	Date of Study	Place of Study	No. of Cases Examined	Percentage of	
				Positive Seroreactions	Non-specific Reactions
Turunen (7)	1926—1934	Maternity Health Center, Helsinki	2,390	2.2 WR or Kahn or both	0.75
Honkanen (1)	1935	Tuberculosis Hospital, Helsinki	611	5.2 Kahn	0.66
Maijala (3)	1939	Factory, Eastern Finland	527	1.6 WR I 1.6 WR II 2.2 Kahn	
Maijala (4)	1936—1937	Medical Clinic, Helsinki	10,953 10,953 10,925	2.54 WR I 2.84 WR II 3.39 Kahn	WR I 0.04 WR II 0.06 Kahn 0.11
Penttinen (5)	1935—1945	Maternity Health Center, Helsinki	18,090	2.49 WR or Kahn or both	WR 0.03 Kahn 0.5
Leineberg (2)	1948—1949	Factories, Vaasa	5,684	1.09 WR and Kahn	0.19

MATERIAL

Our series comprises all the blood donors during the war years 1939—1944 in Finland. These volunteers were on record at the 20 blood pool centers, where a card was filed for each donor containing his personal data, result of the STS, and other information on his general condition necessary from the point of view of blood transfusion. The STS was carried out at six laboratories (located at Helsinki, Vaasa, Oulu, Kuopio, Viipuri and Petrosavodsk), which were under centralized supervision and consequently employed the same technique. In general the Kahn reaction alone was considered sufficient, and only in exceptional cases were other tests made. Therefore in examining the material we are mainly dependent upon Kahn reaction and employ the term Kahn positive instead of STS positive.

The series under consideration is not a random sample of the whole nation but forms a selected group. Most of the volunteers

were young and judging from the appearance healthy individuals;
the majority were women. Moreover, the various occupations are
represented in our series in proportions different from those in
the whole population, and the same is the case as to the marital
status.

The series comprises 81,795 persons, of whom 1,131, or 1.38 per
cent, gave positive or doubtful reactions. The reaction was strong
in 823 and weak in 304 cases. In 4 cases the strength of the reac-
tion is not stated.

Owing to lack of clinical examinations we cannot judge the
specificity of the positive reactions. However, Penttinen's investi-
gation gives us some idea of this subject. He states that 98.8 per
cent of the strong Kahn reactions obtained by the laboratory tech-
nique used in Finland are cases of syphilis, likewise 57 per cent of
the weak reactions if —? are included, as in the present paper.
Penttinen's series, it is true, is composed of pregnant women,
but he convincingly proves that pregnancy in itself is not the
cause of nonspecific reactions but that they are due to other
factors. If we apply his percentages to our present series we find
that syphilis was present in 999 cases, or 1.22 per cent of all
persons examined.

AGE AND SEX

Kahn positive blood donors are classified according to age and
sex in Table 2.

We note that the incidence of positive reactions is relatively
low in the youngest age groups. It increases in the older groups
and reaches its peak both in men and women at the age of a little
above 40 years, after which it decreases. This peak coincides very
well with Maijala's (4) findings.

The majority of the fresh cases reported in Finland fall in the age
group of 21—30 years, and women are younger than men. Thus
most Kahn positive cases in our blood donor series were con-
siderably older than those who contract fresh syphilis; the women,
who form the majority in our series, were more than 15 years older.
Accordingly the greater part of the Kahn positive blood donors
were at the stage where late neurosyphilis and cardiovascular
syphilis already ravage.

TABLE 2

DISTRIBUTION OF KAHN POSITIVE BLOOD DONORS BY AGE AND SEX

Date of Birth	Age-group	Kahn Positive Donors					
		Male		Female		Total	
		No.	%	No.	%	No.	%
1925—29	17—21	—	—	37	0.6	37	0.62±0.10
1920—24	22—26	6	0.9	231	1.0	237	1.02±0.07
1915—19	27—31	6	0.7	204	1.4	210	1.35±0.09
1910—14	32—36	17	1.2	168	1.5	185	1.48±0.11
1905—09	37—41	19	1.4	136	1.6	155	1.61±0.13
1900—04	42—46	19	1.9	144	2.5	163	2.41±0.19
1895—99	47—51	9	1.3	83	2.2	92	2.03±0.21
1890—94	52—56	1	0.3	28	2.0	29	1.70±0.31
1885—89	57—61	2	1.1	8	1.0	10	1.06±0.33
Unknown		1	0.7	12	1.5	13	1.38
	Total	80	1.2	1,051	1.4	1,131	1.38±0.04

The older a person the greater is the possibility of previously contracted syphilitic infection. The statistical series indicating this possibility is cumulative. The factors which decrease the incidence of positive Kahn reactions are, on one hand, recognition of the disease and treatment and, on the other hand, death, but the effect of these factors does not begin to manifest until at a higher age, as our series demonstrates.

Table 2 demonstrates that the incidence of positive Kahn reactions is lower for men than for women in all age groups. This is apparently ascribable to the fact that syphilis in women more frequently remains undetected than in men.

Maijala's (4) observation is quite the opposite. In his series he found 1.3 times as many cases of syphilis in the men as in the women. The explanation for the apparent discrepancy is doubtless the fact that our material consists of persons considering themselves normal, whereas Maijala's consists of patients consulting a physician. In the latter the incidence of syphilis may indeed be greater in the men that in the women, because statistics of fresh syphilis show throughout a preponderance of male cases.

MARITAL STATUS

The marital status of the donors was reported very poorly. For almost half of the women and for practically all of the men it remains unknown. Among the 21,978 women known to be unmarried the percentage of Kahn positives was 1.1, among the 19,052 married, widowed or divorced women it was 1.7. The difference is due to the different age composition of the two groups.

The incidence of fresh syphilis in unmarried persons is much higher than in married persons. Consequently it is evident that the syphilis in the latter category was very frequently of premarital origin.

OCCUPATION

The low incidence of Kahn positives in the rural population is shown by Table 3, which classifies the material according to occupation. Agriculture has the most favorable rate of all the occupations listed. The incidence of previously unrecognized syphilis is almost as low in persons engaged in public service and the professions, among whom we have been compelled to include also office employees for lack of precise information on their field of work. The great difference between handicrafts and industry, to the credit of the former, as well as the high incidence among domestic servants, which approaches that of industrial employees, are to be noted.

For the sake of comparison, Table 3 also lists the cases of fresh syphilis reported in 1945 in relation to the occupational breakdown of the entire population in 1940. As these two occupational breakdowns are derived from two different sources, the classification cannot be fully consistent and consequently the percentages computed also may show some bias. It seems that the physicians have included a relatively great number of workers of various kinds in the common laborer group, whereas the official population statistics include them in the categories of industry and handicraft. The result is a disproportionately high incidence of fresh syphilis in the common laborer group at the expense of the industrial and handicraft workers.

TABLE 3

OCCUPATIONAL DISTRIBUTION OF KAHN POSITIVE BLOOD DONORS AND OF OFFI-
CIALLY RECORDED CASES OF FRESH SYPHILIS

Occupation	Kahn Positive Blood Donors	Cases of Fresh Syphilis in Whole Finland 1945
	%	per 1,000 persons
Agriculture	0.70 ± 0.10	0.66
Public service and the professions ..	0.81 ± 0.07	2.70
Handicrafts	1.34 ± 0.18	[1]
Commerce........................	1.73 ± 0.12	3.87
Traffic	—	6.91
Servants	2.05 ± 0.18	—
Industry	2.26 ± 0.14	3.30[1]
Common laborers	––	11.76

[1] Handicrafts included in industry.

Upon examining the above table with due regard to the reservations stated we observe that both the cases of fresh syphilis and the Kahn positives among the blood donors are distributed approximately similarly among the occupational groups. We must, however, stress as a typical phenomenon that unrecognized syphilis as compared with reported cases of fresh syphilis is rare among the educated classes, the latter being represented primarily by the professions and public service.

A more detailed occupational classification reveals a number of interesting facts. Thus classified, the material comprises rather small groups and we have therefore tabulated them in Table 4 according to the degree of certainty in the deviation of the incidence of Kahn positives from the mean value for the respective sex in the several occupations. The deviation of the incidence from the mean is regarded as certain if it is not less than three times its standard error, highly probable if it is two times the standard error, and probable if it is at least equal to it.

On examining the figures for the women we note that the Kahn positive frequency was highest in the masseuses and steambath attendants, but this may be a mere chance due to their small number. The textile and clothing industry workers, common laborers in population centers, and restaurant kitchen staff also stand out;

TABLE 4

CLASSIFICATION OF THE MATERIAL ACCORDING TO OCCUPATIONS AND KAHN
POSITIVE REACTIONS

Entire Series	
Male (Mean 1.18±0.13%)	*Female* (Mean 1.40±0.04%)
Certainly higher than the mean	
	Textile and clothing work- ers .... 3.58±0.56 Restaurant kitchen staff .. 2.44±0.32 Common laborers in popu- lation centers .... 2.26±0.18
Very probably higher than the mean	
	Masseuses, steambath at- tendants .... 6.29±2.03 Barbers, hairdressers.... 3.26±0.72 Chorewomen .... 2.56±0.51
Probably higher than the mean	
Warehousemen .... 2.80±1.38 Railroad and streetcar male personnel ...... 2.35±0.77 Shopkeepers .... 2.09±0.73	Laundresses .... 3.43±1.02 Railroad and streetcar fe- male personnel .... 3.30±1.32 Domestic servants ...... 1.70±0.20
Approximately equal to the mean	
Skilled labor .... 1.40±0.40 Common laborers in popu- lation centers .... 1.20±0.34	Waitresses.............. 1.74±0.35 Dressmakers .... 1.47±0.25 Shopkeepers .... 1.32±0.42 Dairymaids .... 1.10±0.37
Probably lower than the mean	
Farming population .... 0.53±0.37	Saleswomen .... 1.15±0.15 Handicraft workers excl. dressmakers .... 0.99±0.27
Certainly lower than the mean	
	Office staff .... 0.99±0.10 School pupils............ 0.71±0.18 University graduates .... 0.64±0.20 Farming population .... 0.63±0.11 Medical staff .... 0.50±0.15 University students .... 0.34±0.14

their Kahn positive frequency is, odd enough, much higher than that of the waitresses. Other details will be seen from the table.

Owing to the paucity of the male material, comparison between the occupational groups sheds but little light on the question.

Another interesting aspect is the variation in the geographic distribution of the incidence in the social classes. We divided the localities into two groups, i.e. those of relatively high incidence and those of relatively low incidence. The former group consists of Lahti (2.86 per cent), Kuopio (2.80 per cent) and Tampere (2.09 per cent), and the latter of Rovaniemi (0.54 per cent), Savonlinna (0.49 per cent), Oulu (0.35 per cent) and Jyväskylä (0.16 per cent). The comparison between these two groups presented in Table 5 exhibits the noteworthy phenomenon that the frequency distribution of the social classes in the low incidence localities shows no marked variation, and taking into consideration the standard errors these variations are not at all certain. Difference in social status is a significant factor only where the incidence is high. No locality or social class is free from Kahn positives, but in the localities with an alarmingly high incidence it is the working class and circles near it which form the peak. In comparing the best localities with the worst we note that in the latter the incidence is hardly twice as great in the educated class and three times as great in the farming class, whereas it is seven times as great among the business staff and eight times as great in the working class.

TABLE 5

DISTRIBUTION OF KAHN POSITIVES ACCORDING TO SOCIAL STATUS IN THE BEST
AND WORST LOCALITIES

Social Status	Percentage of Kahn Positives in Entire Series		
	Best Localities	Worst Localities	Total Material
Educated class	0.34	0.64	0.48
Farming class	0.41	1.21	0.63
Foremen, etc.	0.19	2.57	1.06
Business staff, etc.	0.28	2.06	1.27
Labor class	0.44	3.45	2.04
Others and unknown..........	0.31	2.23	1.23
Total	0.35	2.51	1.38

SUMMARY

It was found that among 81,795 blood donor volunteers in the period 1939—1944, the majority of whom were women, 1.38 per cent gave a strongly or weakly positive reaction to the Kahn test. Probably about 1.22 per cent were cases of syphilis, and the majority of persons involved were unaware of their disease. The incidence of Kahn positives was higher in the female than in the male blood donors, and greatest at the age of over 40 years, that is to say, in an age group more than 15 years older than that of fresh syphilis.

In evaluating various occupations great and interesting differences were observed. In comparing the incidence of Kahn positives with the statistically recorded cases of fresh syphilis, we note as a typical phenomenon that the former are extremely rare in the educated class as compared with the reported cases therein, whereas in the labor class they are very frequent. These variations according to social status are heightened where the incidence is high. No locality nor social class is free from unrecognized syphilis, but in localities where the incidence is high it concentrates on the working class and the circles close to it. In going over from the best localities to the worst, the incidence of unrecognized syphilis is hardly twice as high as in the best localities in the educated class, three times as high in the agricultural class, seven times in the business staff, and eight times in the labor class.

REFERENCES

1. HONKANEN, A.: Acta Soc. Med. Fenn. Duodecim (Fld.), Ser. A., Tom. 19, Fasc. 1, 1936.
2. LEINEBERG, O.: Vasabladet, June 29th, 1949.
3. MAIJALA, P.: Duodecim (Fld.) 1939:55:557.
4. MAIJALA, P.: Klinische Untersuchungen über die Häufigkeit und Art der seropositiven Spätlues in Finland. Diss. Helsinki, 1942.
5. PENTTINEN, K.: On the Wassermann and Kahn Reactions during Pregnancy. Diss. Helsinki, 1946.
6. RENKONEN, K. O.: Sotilaslääketieteellinen aikakauslehti 1946:1.
7. TURUNEN, A.: Duodecim (Fld.) 1935:51:831.

HYDROMECHANICS OF DIFFERENT TYPES OF KIDNEY PELVIS

By

ANTTI PAALANEN

Although the hydromechanics and function of the kidney have been frequently investigated, no detailed studies of the kidney pelvis in this respect have come to my notice in the literature I have seen. Therefore this study of the hydromechanics of the different types of renal pelvis seemed desirable.

Among the different morphological classifications Jastrzebski's (4) is so clear and accurate that it was selected as a basis for this study: he distinguished three main types of renal pelvis with different subgroups, and their morphology appears in the attached drawings. It is of interest that nothing can be said with absolute accuracy concerning the mechanism of the different currents and eddies in the kidney pelvis because it is dependent upon many factors, e.g. on the resilience of its wall, possible movements of the pelvis itself, the width of the uretero-pelvic juncture, the degree of diuresis, and possibly on the form of the pelvis.

Two points in the renal pelvis have received much attention from the point of view of hydromechanics, i.e. the calix renalis and the uretero-pelvic juncture. Haebler (3) observed peristaltic waves, 4 to 5 contractions per minute in the calix renalis of the cat, but he was not able to demonstrate similar movements in the human kidney pelvis. According to Lauber and Scherer (7) peristalsis does not occur in the whole renal pelvis (anatomical pelvis + calices); there are only rhythmic contractions. Jona (6) has shown that apparently the m. spiralis papillae, described — but incorrectly

Type I   Type IIa   Type IIb   Type IIc

Type IId   Type IIe   Type IIIa   Type IIIb
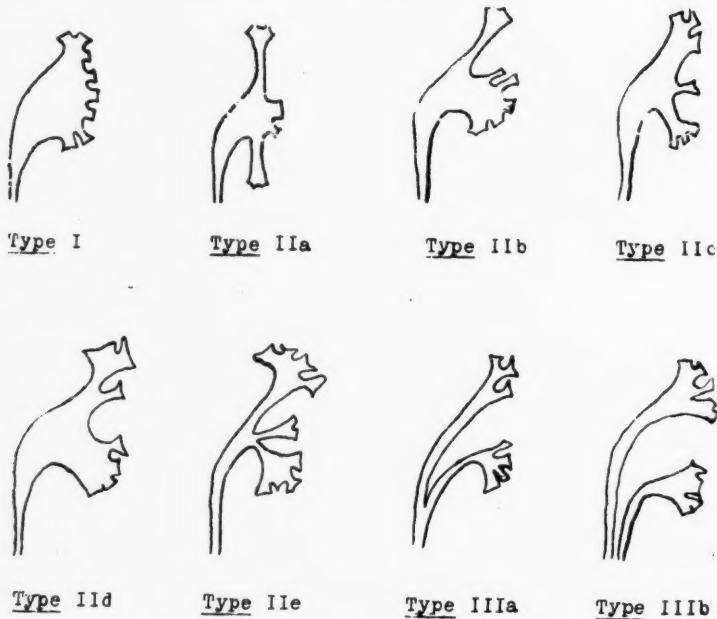
The types of renal pelvis (Jastrzebski).

named from the topographical point of view — by Muschat (8), consti-
tutes the fixed point at which the contraction of the calix begins.
When the systole of the calix is complete, the renal pelvis, until then
relaxed, empties with a rapid contraction. In his study of the
function of the calix (calix minor) Narath (9) divides this into
two parts: the collecting phase (the m. sphincter calycis contracts)
and the emptying phase (the m. sphincter calycis is relaxed).
Potential formation of eddies even in the calix may be due to this
mode of functioning and to the angle between the calix and the
renal papilla (2). According to Narath, the uretero-pelvic juncture
opens when the m. sphincter calycis contracts but this relationship
is only physiological: anatomically there is no m. sphincter pelvis,
as demonstrated also by Jewett (5). Generally the transition from
renal pelvis to ureter is gradual without any sharply defined
limit (2, 5).

For these investigations glass models of the renal pelvis, about
natural size, and the apparatus shown in the Fig. 1, were used.
The solution employed in the tests was olive oil emulsion, specific
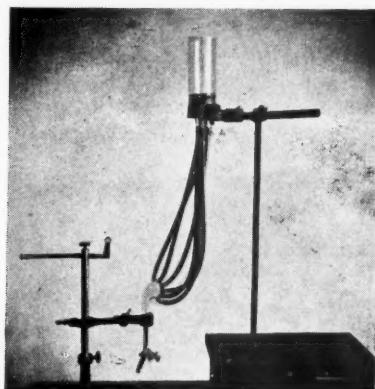
Fig. 1

gravity 1,006, and this proved very suitable especially from the point of view of photographic technique. The glass pelvis was first filled with water and then olive oil emulsion was allowed to flow into it from a glass cylinder. Because of its slow movement and whiteness the emulsion gave a clear idea of the hydromechanics of the different types of glass models, and this could also be demonstrated in photographs. *Throughout the test the pressure was maintained at the level of secretory pressure, at about 30 mm Hg according to Narath (i.e. 40.5 cm emulsion).* A rubber tube, corresponding to the ureter, was the outlet through which the fluid could escape at the rate of one drop per 5 seconds; thus the stream through the glass pelvis continued. The inner diameter of the rubber tube was 4 mm — the average inner diameter of the ureter. In the different types of pelvis the currents in the upright position were as follows:

Type I. — The currents coming from the calices first travel some distance into the glass pelvis and then begin to move nearer the side of the calices and turn towards the ureter. When the currents reach the lower part of the pelvis, there arise upward currents starting from the uretero-pelvic juncture and passing along the medial side, i.e. where the stream directed outwards to the ureter is weakest. This upward current now travels towards the side of the calices and there joins the primary current. Thus there is a permanent eddy in the central and medial parts of the model pelvis.

Among the subgroups of Type II, only the most usual, II a, II b and II d, were studied. Types II c and II e are very uncommon
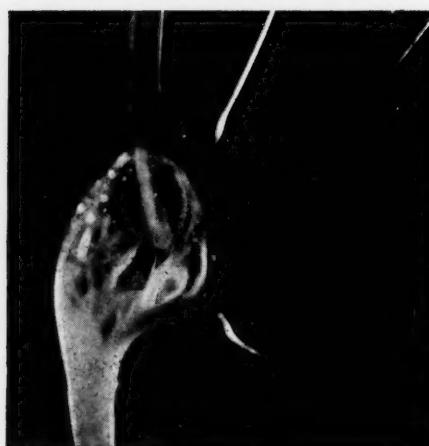
Fig. 2 — Type I
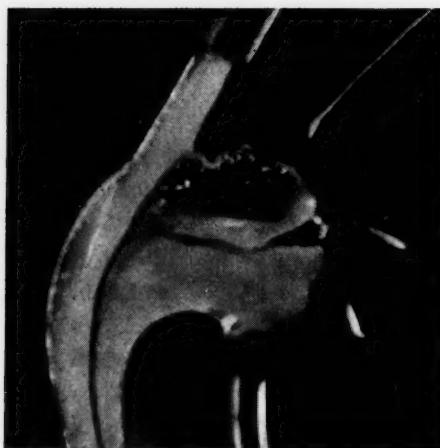
Fig. 3 — Type II (b)

compared with the others (10). Type III a, in which the ureter is still undivided, was selected to represent Type III.

Type II a. — The currents from the lateral calices pass obliquely across the glass pelvis towards the ureter. A downward stream from the upper calix flows near the calices and joins the lateral currents. The lower part of the glass pelvis is gradually filled with fluid, to which currents from below also contribute. But then upward
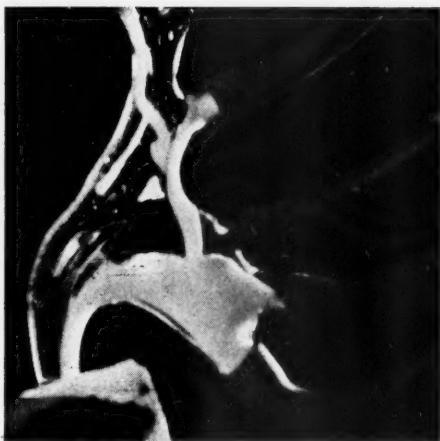
Fig. 4 — Type II (d)

Fig. 5 — Type III (a)

currents gradually appear along the medial aspect of the pelvis. These rise to the mouth of the upper calix and there turn towards the primary current. Thus a permanent eddy is set up practically in the middle of the pelvis; the emulsion surface does not rise to this area but remains in the lower part of the pelvis.

Type II b. — As it reaches the area of the pelvis the current from the uppermost glass calix turns towards the side of the glass

calices on its way to the ureter. This current, too, shows a tendency to approach the side of the calices. From the lower calices upward currents ascend and meet the downward flowing streams. The lower part of the pelvis is gradually filled with fluid. Part of the currents from the lateral calices unites with the upper stream, and part flows past it and turns upwards along the medial side of the glass pelvis, as does part of the current coming from above. This upward stream forces the currents originating from the calices gradually nearer the calices and so an eddy is produced in the upper and medial parts of the pelvis.

Type II d. — In this type the currents from the uppermost glass calices also show a distinct tendency to approach the side of the calices. The current from the upper lateral calix runs downward with those mentioned above, but part of it passes by them and turns up along the medial side of the glass pelvis. When this current has continued for some time an upward stream is set up also at the uretero-pelvic juncture. The upward currents reach rather high up, as far as to the mouth of the lateral calix. As they meet the currents coming from above they push these gradually nearer the side of the calices. Fluid collects in the lower part of the pelvis and the current from below also helps to fill it. Thus a distinct eddy is produced in the central and medial part of the pelvis, just as in the types described already.

Type III a. — In this uncommon type no clear kidney pelvis is distinguished: it is divided into two parts. In the secondary pelves, especially the upper, there is a small intercalycine eddy. Otherwise there is a regular flow without eddies toward the ureter.

DISCUSSION

The different currents travelling through the kidney pelvis depend upon many factors, for instance on the resilience of its wall and possible movements of the pelvis itself. In spite of this fact glass models have been used in this study of the hydromechanics of the different kidney pelves. The types selected for this work are commonest of the three main types found in human subjects (Paalanen). The hydromechanics of these different glass models

were rather regular and coherent and characterized especially in the morphological types I and II by a dual direction of currents and consequent eddy-like formations.

SUMMARY

In this investigation of the hydromechanics of the different types of kidney pelvis glass models of the renal pelvis about natural size were used. Olive oil emulsion (sp.gr. 1,006) was used for these experiments which were carried out under a pressure of 30 mm Hg (corresponding to 40.5 cm emulsion). The results were as follows: In the glass models corresponding to the morphological types I and II the streams coming from the glass calices show a tendency to approach the side of the calices. Uppward-flowing secondary streams begin to separate from the primary currents at the uretero-pelvic juncture. They travel along the medial aspect of the glass pelvis, i.e. where the upward current is weakest, and when they meet the primary currents they push them gradually nearer the side of the calices. These currents set up an eddy in the medial part of the glass pelvis. Thus the currents and eddy-like formations in these types are rather similar and coherent. In the type III glass model the current is regular and free of eddies, except for a small intercalycine eddy in the secondary pelves.

REFERENCES

1. FUCHS, F.: Z. Urol. Chir. 1926:*21*:201.
2. FUCHS, F.: Z. Urol. Chir. 1931:*33*:1.
3. HAEBLER, H.: Z. Urol. 1922:*16*:145.
4. JASTRZEBSKI, C.: Sur la variabilité des calices rénaux. Kosmos Rocz-nick L. I. Za rok 1926. Lwow. 1927.
5. JEWETT, H.: J. Urol. 1940:*44*:247.
6. JONA, L.: Surg. Gynec. & Obst. 1931:*53*:644.
7. LAUBER, H., and SCHERER, F.: Forts. a. d. Geb. d. Röntgenstrahlen 1940:*61*:222.
8. MUSCHAT, M.: J. Urol. 1926:*16*:351.
9. NARATH, P.: J. Urol. 1940:*43*:145.
10. PAALANEN, A.: Ann. Chir. et Gynaec. Fenn. 1949:*38*:134.

ON THE CAPILLARY NET OF THE HUMAN CEREBRAL HEMISPHERES DURING THE EARLY FETAL PERIOD

By

KALEVI NIEMINEVA

· A defective development of the capillary network is regarded by Mali and Räihä (1) as one of the causes of exceptional vital processes occurring in premature infants. However, accurate data on this development in the fetal period of man are still scarce, particularly with regard to the central nervous system. Phylogenetically a strong development of the cerebral hemispheres is characteristic of man. Their growth is stronger, in relation to other parts of the brain, from the third to fifth fetal month, during which period the cerebral hemispheres grow over the parts of the brain lying behind them (2, 3). It was my purpose to investigate whether this period of strong development of the organ is also associated with an increased density of the capillary net, since no such study has been made before in relation to man.[1]

It is comparatively easy to follow the development of the capillary net in the cerebral hemispheres during the fetal period already mentioned. The hemispheres do not as yet reveal any distinct structure; the grey cerebral cortex is in the process of formation. Although the lateral fissure develops in the course of this period, the general formation of fissures has not yet begun. It has been demonstrated that in rabbit embryos of 16 days' gestation and in cats of 8 cm the capillary richness in the cerebral

---

[1] Publication of my report was encouraged by Prof. C. E. Räihä, M.D.

hemispheres is similar throughout (4). Craigie, a pioneer in research on capillaries of the brain, has established that in the newborn rat the capillary network is not only much poorer than at maturity, but the differences in capillary richness among the various parts are less marked at birth than later in life (5). It has been found that in the human brain stem capillary bundles become smaller in the grey than in the white matter only towards the end of fetal life (6).

MATERIAL AND METHODS

The investigation was performed on 12 human fetuses which Professor A. Turunen has kindly placed at my disposal at the Women's Clinic of the University of Helsinki. The weight and length of the fetuses (registered immediately after birth, before fixation) are shown on the table. The thickness of their cerebral

TABLE

Fetus number	Weight in Grams	Crown Heel Length (cm)	Capillaries in Centimeter Length per Cubic Millimeter		Average Diameter in μ		Volume in cu. mm of Capillaries in cu. mm of Tissue	
I. 122	8.5	6.5	6.2		6.2		0.002	
216	15.0	8.5	5.2	6.9	6.5	6.7	0.002	0.002 (5)
221	17.0	9.0	8.3		7.2		0.003	
75	24.0	10.0	7.9		6.8		0.003	
II. 62	65.0	12.0	6.5		7.7		0.003	
63	68.0	12.0	5.3	7.6	8.0	7.7	0.003	0.003 (5)
77	90.0	16.0	9.1		6.8		0.003	
113	90.0	16.0	9.5		8.4		0.005	
III. 78	120.0	18.0	8.4		7.9		0.004	
84	130.0	18.0	11.9	10.7	8.4	8.8	0.006	0.006 (5)
74	330.0	27.0	12.2		9.8		0.009	
65	370.0	28.0	10.3		9.0		0.007	

hemispheres (in sections) in the region of investigation was 0.4 — 0.6 mm for the smaller, and 5—6 mm for the larger fetuses. The original series consisted of 30 fetuses, but the ultimate table comprises only 12, which stained best. It must be observed that this

best eliminates the possibility of loss of blood from the capillaries due to an unsuccessful removal of the specimen or for some other mishap of preparation as well as any lack of blood due to physiological causes. On the other hand, Sjöstrand has demonstrated that the variation of the amount of peripheral blood under different conditions is smaller in the brain than in all other organs (7).

Fixation and staining were performed in accordance with Sjöstrand's original benzidine method (8). Since the study does not make any differentiation of the different layers of cerebral hemispheres, tissue staining was deemed unnecessary. Autopsy and fixation following it were performed in several cases immediately after death (therapeutical abortion), in any event not later than within 24 hours. After the opening of the skull the fetus was immersed for 24 hours in a 10 per cent formalin solution, and the specimens were taken after this. Where smaller fetuses were concerned, the samples were taken by a one stage procedure. They were withdrawn from both hemispheres from the lateral fissure in the fronto-parietal region up to the longitudinal fissure. The sections had a thickness of 100 u. The section was made at right angles to the hemisphere surface.

The capillaries (red blood cells) stained well. Especially with a view to demonstrating the thin capillaries of the fetal period, the statement made by Lindgren in connection with his extensive studies on capillaries: »Das eigene Blut ist die beste Injektionsflüssigkeit» appears fully justified (9).

The actual measurements were performed only on the left hemisphere, since microscopic examination did not reveal any notable differences between sections taken from either side.

The length of the capillaries of the specimens was measured by projecting the picture (magnification $\times$ 200) on paper and dividing it into squares of 25 cc each, in which the vessels were drawn. The number of the squares of each section studied was 20; five consecutive sections of each specimen were measured. The average value was calculated from the results obtained. In the respective sections the diameters of 50 capillaries were measured (ocular net micrometer, Nochet $\times$ 12, magnification $\times$ 480) and the average value was calculated.

The capillary length was calculated in centimeters per cubic millimeter as done by Lindgren in Sweden in his investigations of the capillary network of the cerebral cortex in man (9). In the same way as he did for obtaining the measurement of the capillary from the two-dimensional value to a three-dimensional value, the resultant measurement was multiplied by $\sqrt{2}$; in the sections the capillaries vary in their course from running perpendicular to the microscope's optic axis to parallel with it. This was done in order to obtain an approximate point of comparison to this work from his values. (His sections were 200 μ). The volume of the capillary network is obtained by using the measurements for the lengths and diameters obtained for the capillaries.

Since it is the aim of this investigation to make a comparative study of the changes in capillary density during the fetal period, shrinking has been entirely disregarded, particularly as attemps have been made to perform the opening, fixation and staining under as uniform conditions as possible.

Finally I wish to stress *that all the figures supplied by me should be regarded as relative values,* i.e. they are only significant as criteria for comparing them to each other within the limits of the present study.

RESULTS AND CONCLUSIONS

Even a most superficial microscopic study of the sections reveals at once the unmistakable development of the capillary network and its increasing richness in the fetal period under investigation. Microphotographs 1, 2, and 3 illustrate this development (Figs. 1, 2, and 3). The general structure of the capillary network begins to assume, in the course of this period, individual features in different parts of the hemispheres. However, this will not be discussed here. In assessing my results, I have regarded the entire hemisphere as one region.

The table shows the fetuses divided into three groups according to their weight. The classification is somewhat arbitrary, but my main objective was that the groups should correspond to the third, fourth and fifth month of fetal life (2). The table makes it clear that the capillary length per tissue unit increases about by half (ca 55 per cent) from the first group to the third. The capillary length calculated in centimeters per cubic millimeter increases $6.9 \rightarrow 10.7$.

The diameter grows, accordingly, by somewhat less than one third. The capillary volume, whose value is the best indicator of the engorgement of the tissue, increases to at least twice its original figure during the period under investigation. The great individual variations in different groups are a striking feature, these variations are further enhanced by the circumstance that all physiological factors cannot be fully eliminated in man. In this respect variations in capillary length are greater than in capillary diameter.

It is concluded that the rapid growth of the cerebral hemispheres in early fetal life has its counterpart in the considerable increase of capillary density and volume. This should be interpreted as a mark

Fig. 1. — Photomicrograph of parietal region of the left cerebral hemisphere. Fetus weighing 8.5 g, crown heel length 6.5 cm. Frontal section, 100 u. Sjöstrands benzidine-staining. × 200.
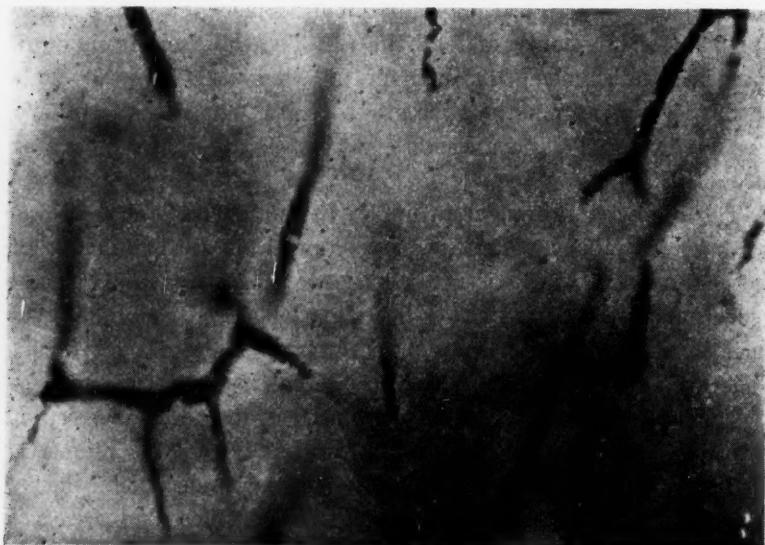
Fig. 2. — Photomicrograph taken from same region under the same magnification as in Fig. 1. Fetus weighing 90 g, crown heel length 16 cm.

Fig. 3. — Photomicrograph from same region under the same magnification as in Fig. 1. Fetus weighing 330 g, crown heel length 27 cm.

of a histologically demonstrable simultaneous development and differentiation of the nervous tissue. The varying capillary density in different parts of the brain in adult life has therefore been interpreted as a criterion for assessing their functions at different stages (5, 10).

Since this study deals with early fetal life, no direct conclusions can be made as to whether or not the viewpoint of Mali and Räihä already mentioned is justified. However, the greater individual variations with regard to capillary length and diameter demonstrated by me in this study seem to indicate that the theory advanced by these authorities, and according to which an undeveloped capillary network is specifically responsible for some disturbances occurring in the premature, is justified. (These premature infants probably have a poorer capillary development than prematures in general.)

According to Lindgren (it should be borne in mind that he strongly emphasises the relativity of his values), the capillary

length in the adult cervical cortex is on an average approximately 20 cm/cu.mm. One can therefore assume that the length of capillary vessels in the adult human is twice their length per tissue unit in this region in fetuses towards the middle of pregnancy. It should be remembered, however, that capillary density in the grey matter of the adult (the subject of Lindgren's investigations) is considerably greater than in the white matter. Evidently (in so far as Lindgren's figures can be compared to mine), it is in the gray matter that the highly significant increase of capillary density occurs towards the end of the fetal period or after birth. This opinion supports the observations of Mali and Räihä. Animal experiments intended to illustrate this development should be applied to man with reservations, since every species is born after a different process of evolution (5, 10).

Research will be continued on these lines with a view to studying the capillary changes occurring in cerebral hemispheres in later fetal life.

SUMMARY

The writer has made a study on the development of the capillary network in the cerebral hemispheres of man during the third to fifth fetal month, which is the period of strong growth of these parts of the brain. The series consisted of 12 fetuses.

Staining was performed according to Sjöstrand's benzidine method. Accurate measurements were carried out in the left hemisphere. The author concludes that:

1. The capillary length per tissue unit increases by half during this period.
2. The diameters of the capillaries increase by one third.
3. The capillary volume per tissue unit is doubled.

REFERENCES

1. MALI, A. M., and RÄIHÄ, C. E.: Acta Paed. 1936:*18:*118.
2. AREY, L. B.: Developmental Anatomy. Philadelphia and London. 1945.
3. BRANDT, W.: Lehrbuch der Embryologie. Basel 1949.
4. VALENTI, G., and D'ABUNDO, G.: Quoted by CRAIGIE, E. HORNE: The Proceedings of the Association for Research in Nervous and Mental Disease 1937:*18:*3.

5. CRAIGIE, E. HORNE: Jour. Comp. Neur. 1925:*39:*301.
6. LUNA, E.: Ricerche di Morfologia 1920:*1:*37.
7. SJÖSTRAND, T.: Skand. Arch. Physiol. 1935:*71:*Suppl.
8. SJÖSTRAND, T.: Skand. Arch. Physiol. 1934:*68:*160.
9. LINDGREN, ÅKE G. H.: Die kapillare Angioarchitektonik der isogenetischen Grosshirnrinde des erwachsenen Menschen. Helsingfors 1940.
10. PETREN, T.: Verhandl. Anat. Ges. Erg. — Heft Anat. Anzeiger 1939: *87:*326.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF
HELSINKI

THE PURIFICATION OF HUMAN ISOAGGLUTININS

By

K. O. RENKONEN

The purification of isoagglutinins and antibodies in human plasma has not given very good results. According to Boyd (1) the concentration of isoagglutinins is 8—10 fold as compared with plasma.

The present writer has attempted a concentration of the iso- agglutinins. A study of the Landsteiner eluates (2) seemed to us more promising than the chemical fractionation of sera. However, when working with these eluates, there is a disturbing factor arising from the hardly avoidable hemolysis of the cells when the agglutinins are eluated at 56°C. The hemoglobin, however, can be completely eliminated if chloroform is mixed with the eluate and the tube is placed in the deep-freeze at −20°C. After thawing it up at room temperature the following morning, all coloured substances are precipitated and can be centrifuged off. The water- clear solution contains the agglutinins with no significant losses. They can be precipitated with 150 per cent acetone at −5°C and resolved in water. According to nitrogen analysis, our purified agglutinin has become 40 times concentrated, on an average, as compared with the serum.

If the Landsteiner eluate is reabsorbed with washed A or B cells respectively, the agglutinated cells are washed, and the agglutinins re-eluated, most of the serum proteins, still present in the first Landsteiner eluate, are removed. This second Land-

TABLE

THE CONCENTRATION OF α-AGGLUTININS OF A B-SERUM (23/10)

Dilutions	B Serum before Absorption [1] with A Cells	B Serum after Absorption with A Cells	First Landsteiner Eluate [2]	Second Landsteiner Eluate after Chloroform Treatment [3]	
	Titrated with A Cells	Titrated with A Cells	Titrated with A Cells	Titrated with	
				A Cells	B Cells
1/1	+ + +	+	0	0	0
1/2	+ + +	+	+ +	+ +	—
1/4	+ + +	—	+ +	+ +	—
1/8	+ + +	—	+ +	+ +	—
1/16	+ + +	—	±	+	—
1/32	+ + .	—	— ?	+	—
1/64	±	—	— (?)	+ ?	—
1/128	— ?	—	—	— ?	—
1/256	—	—	—	—	—
NaCl	—	—	—	—	—

Signs $+$, $++$, and $+++$ indicate increasing strengths of strong agglutination visible to the naked eye. The signs $-(?)$, $-?$, $\pm$ and $+?$ indicate doubtful results, $-(?)$ being the weakest and $+?$ the strongest agglutination. 0 means that the tube was omitted. — The results were read after one hour at room temperature.

[1] Absorption was carried out in an icebox, with one drop of three times washed A cells per ml. The time of absorption was two hours.

[2] There was a reduction here in the volume of serum to ca. 1/10th.

[3] There was a further reduction in the volume to ca. 1/10th of the first Landsteiner eluate. The nitrogen content was here 22 γ/ml.

steiner eluate can also be treated with chloroform at $-20°C$ and yields a highly purified agglutinating solution. The appended table will give the reader a general idea of the procedures. The nitrogen analysis of the chloroform water indicates that the agglutinins from 0 or B serum may be concentrated 400 times on an average and the β-agglutinins somewhat less. From this solution the salts can be removed through dialysis, without loss of agglutinins.

REFERENCES

1. BOYD, W. C.: Fundamentals of Immunology, Second Ed. 1947, p. 48.
2. LANDSTEINER, K., and MILLER, PH.: C. J. Exp. Med. 1925:*42*:853.